ANATOMY OF WASHINGTON NAVEL ORANGE RIND IN RELATION TO WATER SPOT

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Introduction

In southern California the damage caused by water spot to the crop of Washington navel oranges is a cause of increasing concern to the citrus industry (10, 11, 13, 20, 22, 24, 41).

The first visible symptom of injury is a local swelling and waterlogging of the rind in the maturing fruit. The air normally present in the respiratory chamber beneath the guard cells, and also in the intercellular spaces, is replaced by fluid, and, as a result, the surface of the rind becomes raised and more or less translucent. The injury occurs during cold rainy periods in winter, and the progress of the disease appears to depend on weather conditions. If a dry spell follows a rain, the rind swellings may recede within a few days and the fruit remain normal. If, however, the damage has gone too far, the affected areas become sunken, dried out, and brown in color, marking the fruits as culls from the grower's standpoint. If the rain continues, the initial swelling may spread, and, in a matter of hours or days, the epidermis may crack in all directions. Again the weather determines the fate of the fruit. Should the rain cease, the cracked epidermis will heal by normal cicatrization. The rind at maturity will be scarred and shriveled, but the fruit will be otherwise undamaged. On the other hand, if wet weather persists, the ubiquitous spores of molds such as *Penicillium* and *Cladosporium* may enter the cracks, germinate, and spread through and rot the entire fruit within 48 hours.

The localized swelling of the rind tissue in water spot may be classified anatomically as intumescence (26). The behavior of the tissue in *Citrus*, however, differs markedly from that in the intumescences induced in poplar by stimuli as diverse as stagnant moist air and hormone treatment. In poplar the hypertrophy of certain individual cells is exaggerated, whereas in the flavedo cells of *Citrus* the expansion of individual cells is barely noticeable microscopically.

Among the numerous publications on *Citrus*, comparatively few are concerned with the detailed structure of the rind (2, 15, 39, 42). The fruit of *Citrus*, the characteristic hesperidium, consists of carpels, morphologically leaves. The structure of the typical leaf, with its epidermal, photosynthetic, and conducting tissues, is paralleled in the outer epidermis, the flavedo and albedo, and in the vascular network of the fruit. The endocarp or pulp, consisting of juice sacs, is peculiar to the fruit. Oil glands are numerous near the surface in the fruit as in the leaf.

At the present time plant pathologists and physiologists are engaged in the investigation of the causes and possible methods of control of water-spot injury (6). The present paper deals with the anatomy of the rind of the ripening fruit and forms a part of the background necessary for the interpretation of current experimental work.

Material and methods

Material was collected from the university experimental orchards in Los

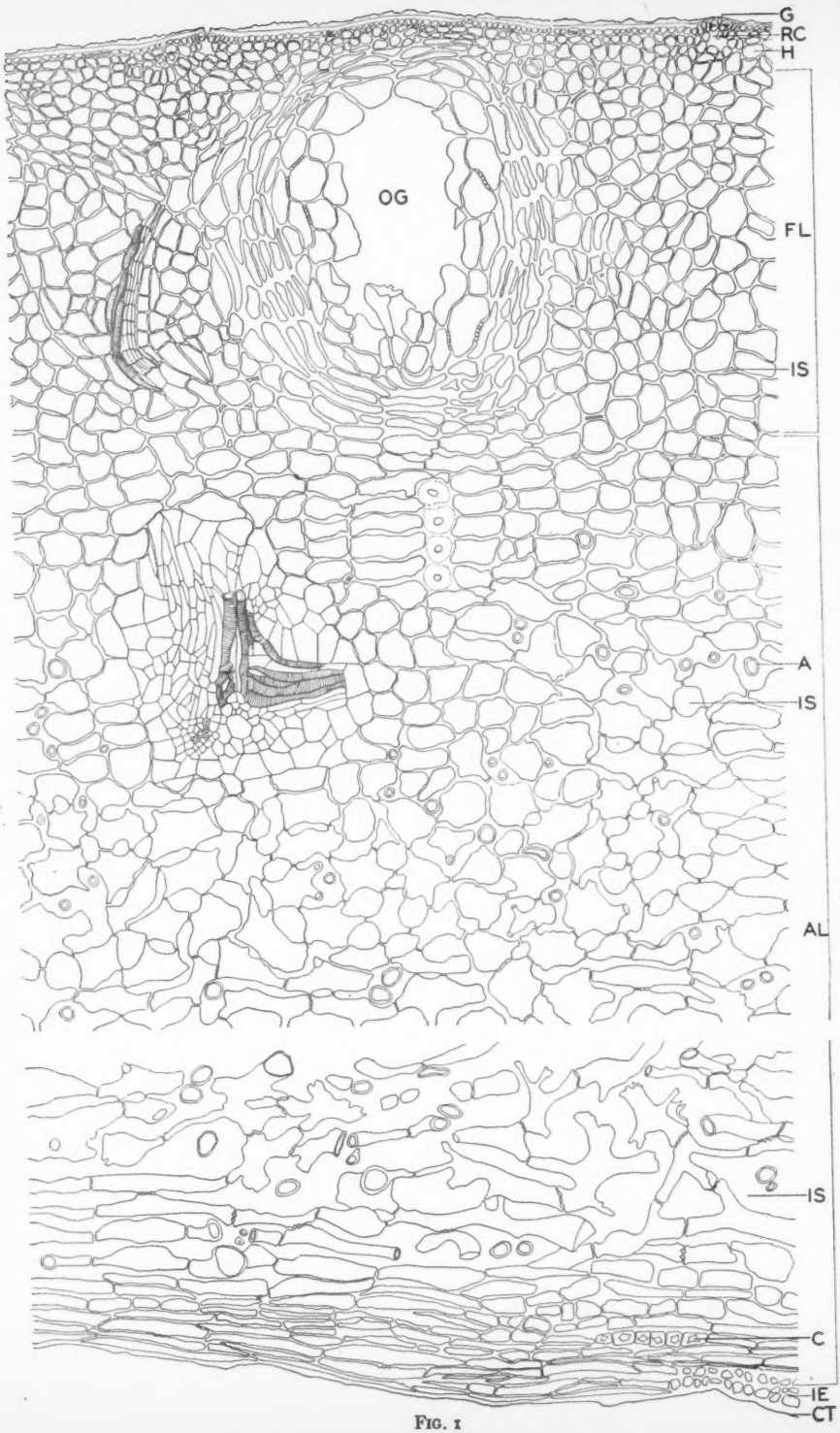


FIG. 1

(Legend on facing page)

Angeles and Riverside and also from the Foothill Ranch, Azusa.[1] The bulk of the fruit had been oil-sprayed at various times of the year in accordance with usual orchard practice. In all groves, however, a number of trees were left unsprayed, so that untreated fruit was available for comparison. In addition, in order to determine the effects, if any, of oil treatment on the anatomy of the rind, growing and picked fruits were dipped in concentrated spray oil.

Fruits in all stages of growth were sectioned transversely, radially, and tangentially. They were examined immediately, and after treatment with the standard microchemical reagents for ergastic substances and wall materials (40). Hesperidin, which crystallizes immediately on dehydration, is present in fruits of all ages and is particularly abundant in the younger.

Plasmodesmatal connections are apparent on application of IKI followed by H_2SO_4 (5%, or stronger if irrigation is used). The results of this method are confirmed by the standard technique of CRAFTS (7).

Wax and cutin are distinguished clearly under the polarizing microscope and also in sections stained with Sudan III or Biebrich Scarlet. In addition to the latter reagents, H_2SO_4 ($\pm$75%) is useful in the study of the cutinization of the respiratory chamber and of the tissues involved in frost injury and wound healing. The radial canals of the outer walls of the epidermal cells appear most clearly after irrigation with IKI with or without the addition of H_2SO_4 ($\pm$5%).

[1] The writers are indebted to Mr. PAUL MOORE for his assistance in experimental treatment of fruit in the field.

In the observation of the vacuoles of living protoplasts, neutral red and methylene blue are effective (18). The latter, because of the more marked color contrast, proved the more useful, but both stains give the same qualitative results.

In the study of albedo development thick sections are essential in determining the main structural pattern of cells and intercellular spaces. Macerated material is necessary in the observation of individual cells.

Observations

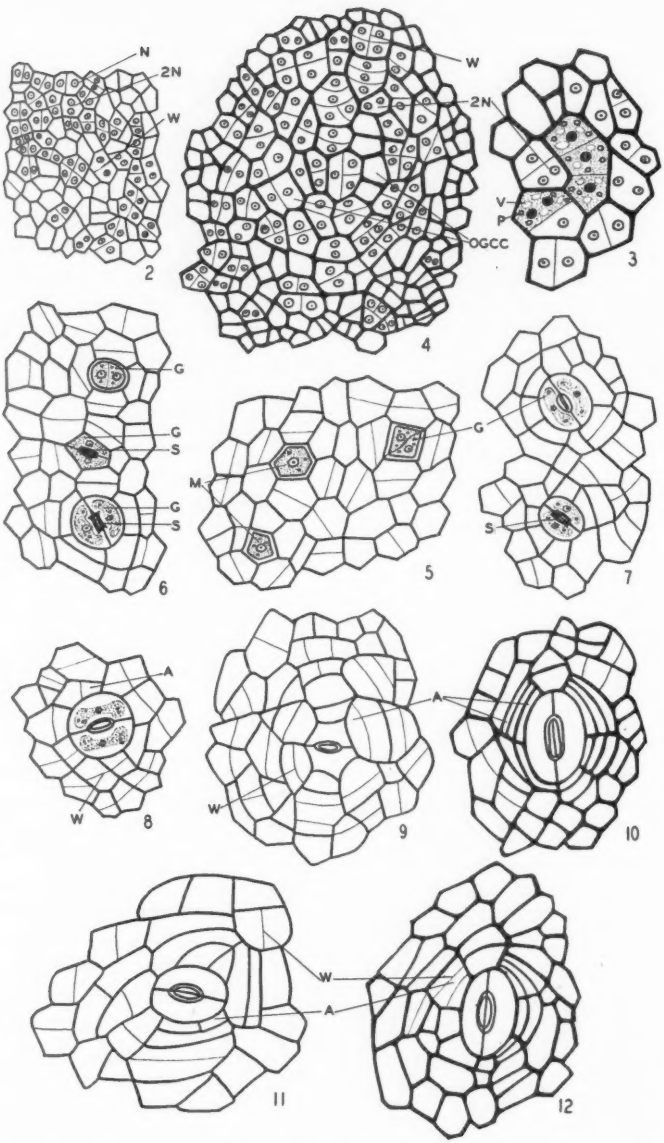
The peel or pericarp, consisting of epicarp, hypodermis, and mesocarp (42), includes the following tissues: epidermis, hypodermis, flavedo, oil glands, vascular bundles, albedo, and inner epidermis (fig. 1). A typical Washington navel pericarp is 8 mm. in thickness; variations from this occur in the albedo tissue.

EPIDERMIS

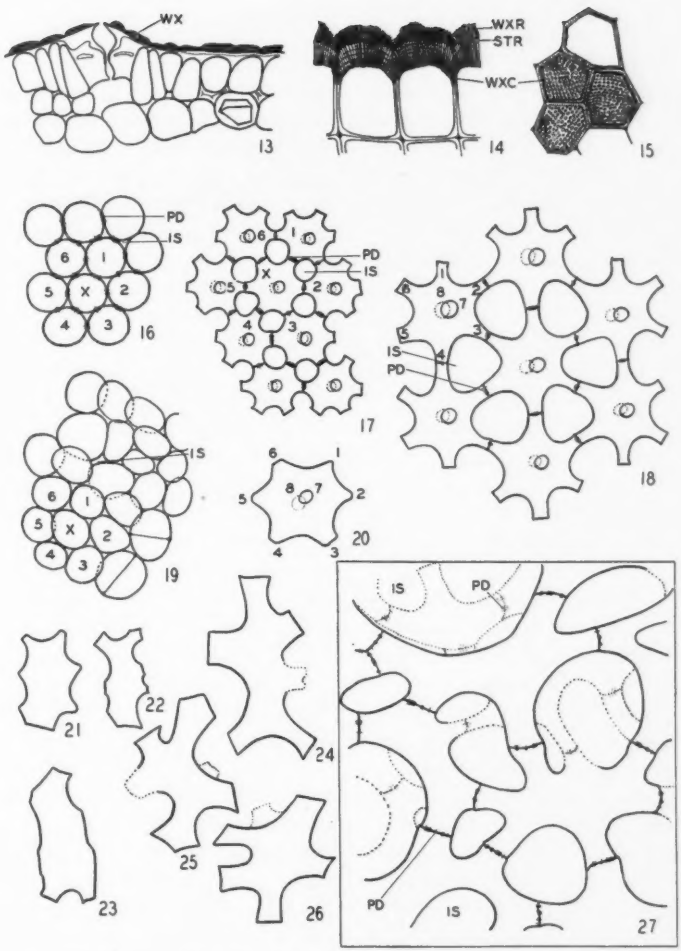
In the epidermis four types of cells may be distinguished: epidermal, guard cells, accessory cells, and oil-gland cover cells (figs. 2–12). Epidermal cells are polygonal in outline and roughly isodiametric. The outer wall, consisting of cellulose and pectic substances, horizontally stratified, is cutinized and further protected by a layer of wax (figs. 13–15). Within the living cells may be identified cytoplasm, nucleus, one or more vacuoles, and plastids. The plastids are approximately equal in size to those of the flavedo, but they are relatively few in number (fig. 50).

Cell division may occur in any region of the epidermis, in the equatorial, stem,

FIG. 1.—Transection of rind of mature fruit 8 mm. from outer to inner epidermis; approximately 4 mm. of mid-albedo has been omitted: *G*, guard cell; *RC*, respiratory chamber; *H*, hypodermis; *FL*, flavedo; *A*, arm of albedo cell; *IS*, intercellular space; *AL*, albedo; *C*, calcium oxalate crystal; *IE*, inner epidermis; *CT*, cutin; *OG*, oil gland.



FIGS. 2–12.—Figs. 2, 3, epidermis, maturing fruit, surface view; cell division, binucleate cells and recently formed thin walls: *N*, nucleus; *2N*, binucleate cell; *W*, new wall; *P*, plastid; *V*, vacuole. Fig. 4, epidermis, maturing fruit, surface view; cell division in oil-gland cover cells: *OGCC*, oil-gland cover cells. Figs. 5–7, epidermis, young fruit, surface view; differentiation of guard cells from mother cell: *G*, guard cell; *M*, mother cell; *S*, stoma. Figs. 8–12, differentiation of accessory cells: *A*, accessory cells; *W*, new wall. Overhang of young accessory cells (fig. 9) is common.



FIGS. 13–27.—Figs. 13–15, epidermis, maturing fruit, transections and surface view: *WX*, wax scale; *WXC*, wax canal; *WXR*, wax rodlet; *STR*, stratification. Figs. 16–18, diagrams, differentiation of eight-armed cells in mid-albedo; a typical cell, **X**, surrounded by eight adjacent cells, arms *7* and *8* at right angles to plane of paper: *PD*, plasmodesmata; *IS*, intercellular space. Fig. 19, mid-albedo of young fruit, transection, camera lucida (compare with fig. 16). Fig. 20, mid-albedo, macerated cell (compare with fig. 18). Figs. 21–23, outer albedo, macerated cells (compare with figs. 28, 29). Figs. 24–26, mid-albedo, macerated cells (compare with figs. 18, 27, 33). Fig. 27, mid-albedo, transection, camera lucida.

or navel zones of the maturing fruit. In fruits considered picking-ripe, binucleate cells lie adjacent to others both older and younger. In the latter the newly formed cell walls may still be barely visible (figs. 2–4). The areas of active cell division are sporadic, patches, streaks, or disconnected networks. They may radiate in any direction from the periphery of large or small oil glands, or may wander between the complex of stomata in the interglandular areas. Since stretching growth necessarily occurs before and after cell division, the comparatively weak external walls of these meristematic cells represent areas of potential cracking.

GUARD CELLS.—The paired guard cells of the stomatal apparatus arise from a mother cell, which at first, in surface view, is distinguished from the surrounding cells only by wall thickening, density of cell content, and accumulation of oil (figs. 5–12). This parent cell is four- to six-sided and is surrounded by the same number of cells of approximately equal size. When cell division occurs, the position of the stoma is soon defined by localized thickenings along the central one-third of the median walls. As the guard cells expand, the stoma widens and becomes elliptical in outline and clearly defined by the cutinized rim (figs. 5–9). In radial section the young guard cells are seen to possess the normal covering of cutin on the outer epidermal surface and on the wall of the stomatal pore (fig. 36). In older stomata cutinization may extend across the inner tangential wall and thence over the surfaces of the cells which line the respiratory chamber (figs. 37–40). By treatment with H_2SO_4, the surface cuticle and the cutinized saclike lining of the respiratory chamber may be isolated. The thickness of the latter ($\pm 2\ \mu$) is less than that of the cuticle of the outer surface ($\pm 3\ \mu$).

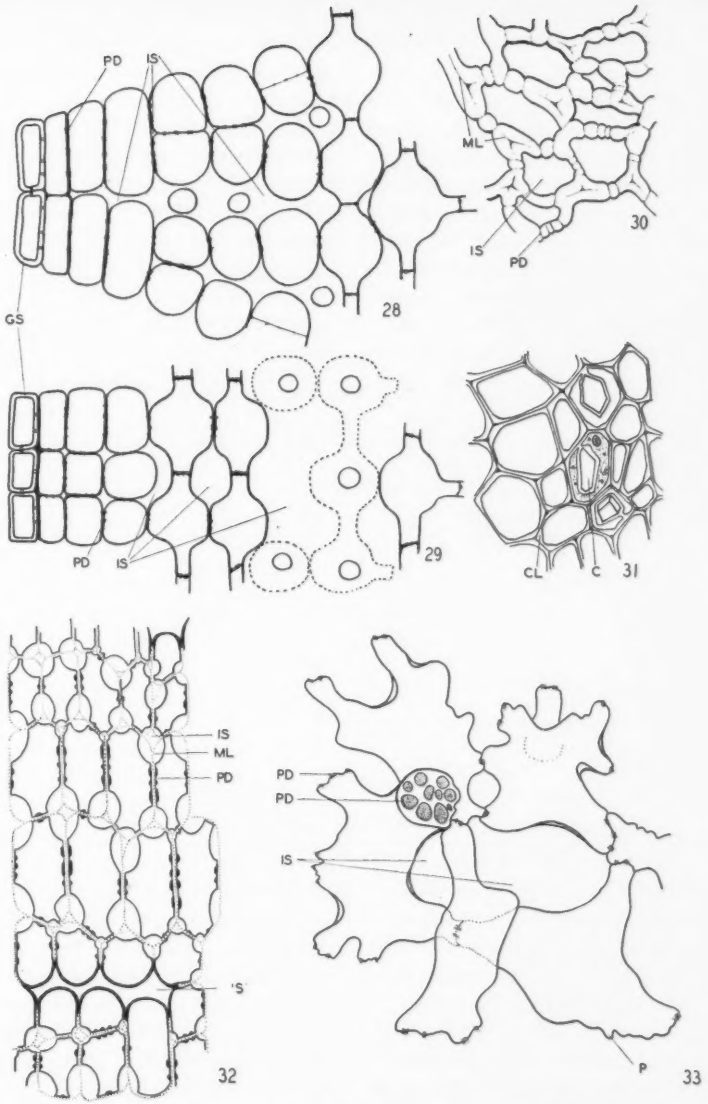
ACCESSORY CELLS.—As the guard cells differentiate and mature, the adjacent cells divide by radial and tangential walls (figs. 5–12). The divisions may be somewhat erratic, but in a typical stomatal area the guard cells are surrounded by from three to four rows of narrow accessory cells. These may extend along six-eighths or seven-eighths of the outer rim of the guard cells while the remaining arc or arcs are occupied by undivided polygonal elements.

In fruits of all ages the surface of the subsidiary cells is comparatively weakly cutinized (figs. 36–39). This may be observed in radial section but is strikingly demonstrated when tangential sections of the epidermis are treated with H_2SO_4 ($\pm 80\%$) (figs. 48, 49). On penetration of the reagent the thinly cutinized walls of the accessory cells immediately swell and thereafter completely disappear, while the heavy cuticle of the isolated guard cells and the rest of the epidermis remains intact.

OIL-GLAND COVER CELLS.—The area of the oil-gland cover cells is proportional to the diameter of the underlying gland (figs. 1, 4). In the mature fruit the cells are somewhat flattened and in surface view polygonal. They may be smaller or larger than the adjacent epidermal cells, since cell diameter varies with the age of the gland. Oil droplets are generally present in the cells, the protoplast of which is further distinguished in tangential section by its comparative translucence.

NUMBER AND DISTRIBUTION OF STOMATA AND OIL GLANDS.—In a recent paper the number and distribution of oil glands and of stomata in the mature fruit have been statistically treated (41).

The repetitive unit in the complex pattern of the rind is the individual oil gland with its orbit of satellite stomata. In a fruit 0.5 cm. in diameter, a typical



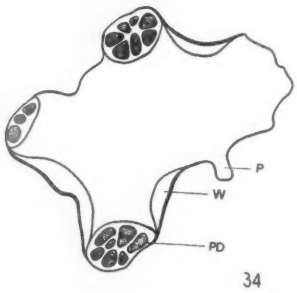
FIGS. 28–33.—Figs. 28, 29, diagrams, outer albedo, transection and radial section; differentiation of branched cells (compare with figs. 16–18, 32): *GS*, gland-sheath cell. Fig. 30, mid-albedo, young fruit, transection; plasmodesmata: *ML*, middle lamella. Fig. 31, hypodermis, maturing fruit, surface view: *C*, calcium oxalate crystal; *CL*, collenchymatous thickening. Fig. 32, outer albedo, developing fruit, transection, camera lucida; early formation of arms and intercellular spaces. Fig. 33, mid-albedo, mature fruit, transection; intercellular spaces, arm and wall plasmodesmata: *P*, papilla.

gland is surrounded by a single orbit in which the stomata, five to eight in number, are approximately equidistant from the gland and from each other. As the gland and, concurrently, the interglandular ridge increase in volume, a second orbit of stomata differentiates. Meantime during the expansion of the fruit, additional oil glands are initiated, each with its potential complement of stomata. Sooner or later, therefore, adjacent orbits intersect, and, as a result, in any interglandular area of the epidermis, stomata of different ages and in varying degrees of development can be identified. The differentiation of stomata is most active in the youngest fruits. It is estimated that, in fruits 1 cm. in diameter, half of the total number of stomata present in the mature fruit are already in process of development. In fruits ± 2.5 cm. in diameter, which are approximately one-third grown, differentiation still continues, and young stages of stomatal development are still of common occurrence. In mature fruits, on the other hand, while cell division persists as already mentioned, all stomata are practically mature.

That cell size increases with age in epidermal and other tissues is shown in table 1.

WAX.—The slight but definite dulness of surface due to the coating of wax is more readily observed on unsprayed than on sprayed fruit. At the time when the groves are normally sprayed, the fruits are approximately two-thirds grown. Oil-spraying does not inhibit the subsequent secretion of wax on the still expanding fruit.

The wax coating consists of a layer of flattish scales roughly equal in area to the varying surfaces of the epidermal cells of all types, including the specialized guard, accessory, and oil-gland cover cells (figs. 13–15). On closer examination each wax platelet is seen to consist of minute vertical rodlets less than 1 μ in diameter and 2–4 μ in length (**8, 9, 29**). In radial section very fine canals are evident in the outer walls of the epidermal cells, the openings of which, when seen in surface view, appear as minute pits. The wax which is synthesized within the epidermal protoplasts, after exudation through the channels, solidifies to form the closely packed and coherent surface



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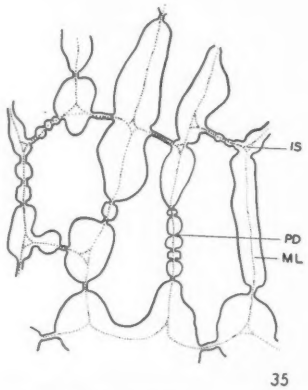

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FIGS. 34–35.—Fig. 34, mid-albedo, mature fruit, individual cells: *W*, curving walls. Fig. 35, outer albedo, developing fruit, transection; early arm and intercellular space formation.

rodlets. The radial canals may also allow of the intermittent transport of the cutinogenic fatty acids, the deposition of which accounts for the increasing thickness of the cuticle.

The thickness of the wax coating in the maturing fruit varies somewhat as might be expected. Buckling and overlapping of scales is doubtless due to the persistence of cell division and consequent expansion of the epidermis. A curious heaping-up of wax platelets is commonly seen in fruits stained overnight in methylene blue. On such fruits there may appear dark-blue, somewhat irregular longitudinal bands, ±3 mm. in width, which may run the entire length of the fruit from stem to navel. Along such streaks the wax scales may be piled up erratically, two or more deep, and methylene blue heavily absorbed by them. The streaks are readily erased by light rubbing. The distribution of the bands bears no obvious relation to segment orientation, or to rind venation, and must therefore result from a basic pattern of growth in the epidermis as a whole.

TABLE 1

SIZE OF TYPICAL CELLS OF WASHINGTON NAVEL ORANGE FRUIT AT DIFFERENT STAGES OF GROWTH AND WALL THICKNESS IN MATURE FRUIT

Typical cell of:	Size of typical cells (μ)				Thickness of walls (μ)
	Fruit 2 cm. diameter	Fruit 3 cm. diameter	Fruit 4 cm. diameter	Mature fruit 8 cm. diameter	Mature fruit 8 cm. diameter
Cuticle	1	1	1.5	3	.....
Epidermis	6×10	7×10	9× 10	11× 15	0.75
Hypodermis	8×10	12×12	13× 15	18× 23	1.50
Flavedo	15×19	21×24	23× 24	40× 50	1.50
Outer albedo	25×30	27×38	30× 64	56× 63	1.00
Inner albedo	27×34	33×60	30×150	34×200	1.00
Inner epidermis	15×55	15×75	15× 75	15× 20–15×100	.....
Epidermis over oil gland	.....	.....	.....	10× 10	0.75
Flavedo over oil gland	.....	.....	.....	15× 25	0.75–1.50
First layer of oil gland, median section	42×75	45×75	45× 75	86×182	1.00
Second layer of oil gland	10×60	25×65	30× 75	50×100	1.50
Third layer of oil gland	12×67	12×67	25× 75	28×100	2.00–8.00
Spiral vessel	.....	.....	.....	8×300	.....

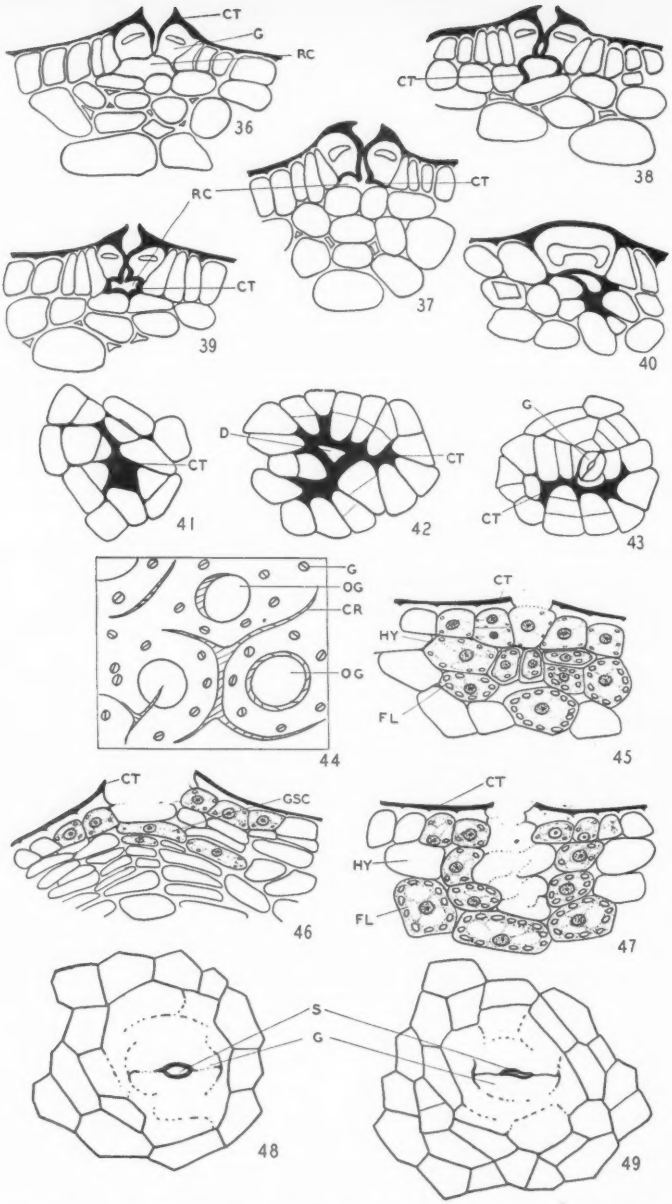
Hypodermis

Beneath the epidermis lies the hypodermis, a somewhat ill-defined layer varying from one to three cells in thickness. It consists of polygonal or ellipsoidal cells with characteristic collenchymatous wall thickenings. Intercellular spaces, although small, are present throughout this tissue in the mature fruit (figs. 1, 13).

Crystal idioblasts are extremely abundant, ranging in number from 350 to 900/sq. mm. (fig. 31). As in the leaf, they encircle, at a distance, both stomatal chambers and oil glands. The earliest stages of development in the youngest fruits are difficult to observe in fresh material owing to the abundance of hesperidin.

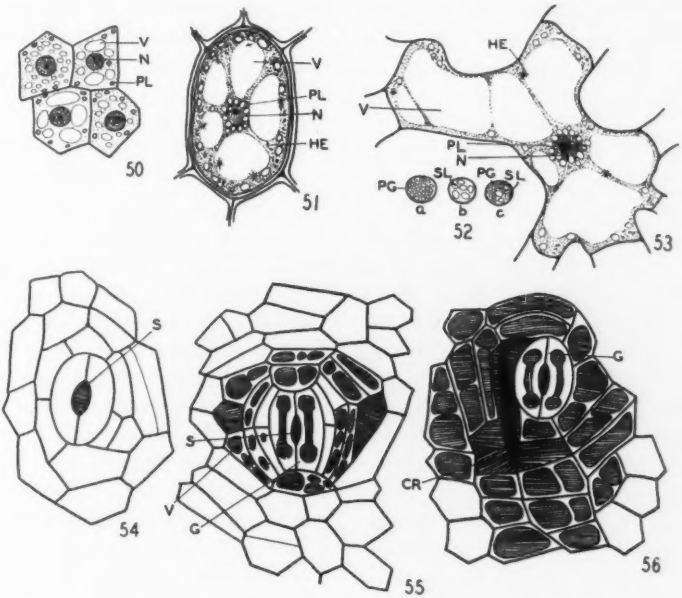
Flavedo

The flavedo is the zone of plastids, oil glands, and vein endings (fig. 1). As in



FIGS. 36–49.—Figs. 36–40, progressive cutinization of respiratory chamber: *CT*, cutin; *G*, guard cell; *RC*, respiratory chamber. Figs. 41–43, epidermis, young fruit, surface view; healing of incipient cracks by cutin: *D*, dead cell. Fig. 44, epidermis, surface view, diagram; pattern of early major cracks: *CR*, crack; *OG*, oil gland. Figs. 45–47, outer rind, transections; progressive cracking: *GSC*, gland-sheath cell; *HY*, hypodermis; *FL*, flavedo. Figs. 48, 49, epidermis, surface view; dissolution of accessory cells in H_2SO_4.

epidermis and hypodermis, cell division continues in the outer flavedo until the fruit is approximately full grown. The cells are polygonal in outline with comparatively small intercellular spaces. Increase in volume of the inner flavedo results from expansion both of cells and of intercellular spaces. The lobed cells of vary. Chromoplasts contain numerous minute granules of pigment, less than 1 μ in diameter. Typical leucoplasts may inclose a varying number of starchlike grains, 1–3 μ in diameter, which stain faintly blue with IKI. Certain other plastids, intermediate in ergastic content, contain both pigment and starch.



FIGS. 50–56.—Fig. 50, epidermal cells, surface view; protoplast: *V*, vacuole; *N*, nucleus; *PL*, plastid. Fig. 51, flavedo cell; protoplast: *HE*, hesperidin. Fig. 52*A*, *B*, *C*, plastids with granules: *PG*, pigment; *SL*, starchlike granules. Fig. 53, albedo cell; protoplast. Figs. 54–56, epidermis, surface view; staining by methylene blue of vacuoles and stomatal plugs: *CR*, crack.

this zone form a transition to the characteristic branched elements of the albedo. The cells adjacent to the oil glands in the outer and inner flavedo are thick-walled, flattened, and characteristically pitted.

Plastids (figs. 51, 52) are abundant throughout the entire tissue, typical chloroplasts in the younger fruits, and chromoplasts in the older. In the mature fruit the contents of the plastids may

Granules and mitochondria are present in the cytoplasm in all flavedo and albedo cells (figs. 50, 51, 53).

OIL GLANDS.—The schizolysigenous development and the structure of the oil glands have been discussed in a number of earlier publications, as have, more recently, their distribution in relation to water spot (**2, 15, 35, 38, 41**).

VENATION.—In the rind, as in the fruit as a whole, the carpel is the unit of struc-

ture. The venation of the carpel is similar to that of the leaf, pinnate-reticulate, with conspicuous midrib and marginal veins (42). The pattern, however, is three-dimensional and may be traced in gross segments of the rind treated with phloroglucin and HCl. The ultimate veinlets bracket the oil glands and extend around them for half of their length (fig. 1). The component elements of the vascular tissue are similar to those of the phloem and xylem of the leaf. The xylem includes spiral vessels and tracheids, reticulate tracheids, and xylem parenchyma (34). As in the leaf the blind veinendings consist of a knot of spiral tracheids only.

Albedo

On first examination of thin sections of albedo, no pattern of structure is distinguishable, but, when development of the tissue is followed in comparatively thick sections, the structural plan becomes clearly defined (figs. 1, 16–27).

Meristematic cells in the young albedo are typical tetrakaidekahedra in form. As they expand, they become spherical, and intercellular spaces appear (figs. 16, 19). Each single cell in this compact tissue is in contact and in direct plasmodesmatal connection with eight other cells of roughly equal size (fig. 18). As growth proceeds in the rind as a whole, the albedo cells fail to sustain the pace of cell division and expansion necessary for the formation of a solid tissue. The intercellular spaces thus expand enormously, and the cell outline becomes more and more deeply lobed, but throughout the entire period of growth the original plasmodesmatal connections are maintained (figs. 17, 18, 34).

With further growth in volume the lobes of the cell extend as arms approximately equal in length. Thus the open mesh of the mid-albedo in the mature fruit consists of typical eight-armed symmetrical cells. Each arm terminates in a sieve-plate or plasmodesmatal area. The symmetry of the cells is evident, since transverse, radial, and tangential sections of the mid-albedo are practically identical in appearance.

Variation in the rate of cell growth in different regions of the rind results in strains and stresses which cause deviation from the above typical pattern.

In the outer albedo, expansion is effected by continuous cell division rather than by increase in cell volume as in mid-albedo. The cells of this outer region differ from the typical eight-armed elements in the shortness of the tangential arms and in the absence of radial ones (figs. 28, 29).

In the inner albedo, cell divisions cease at approximately the same time as in mid-albedo. Arm development in the inner six to ten rows of cells is inhibited by radial compression (fig. 1).

The length of the cell arms in the rind as a whole varies with rind thickness and sponginess. The tissue pattern may be further complicated by the development of adventitious arms. The latter may arise at any point of the cell surface as papillae and may remain short (figs. 33, 34) or extend hypha-like into the intercellular spaces.

Walls throughout the entire albedo are comparatively uniform in thickness. The illusion of collenchyma-like thickenings adjacent to intercellular spaces observed in the necessarily thick sections of fresh material is an optical illusion due to perspective on the curving cell surfaces (figs. 32, 35).

The albedo cells are transparent, and abundant sugar may be demonstrated microchemically within the vacuoles. The peripheral cytoplasm, a thin layer,

extends throughout the arms and thence through the plasmodesmatal connections. The nucleus is generally central in position but occasionally may lie within one of the arms. Plastids are relatively few in number and in younger cells surround the nucleus, while in older cells they are more numerous in the peripheral cytoplasm. Mitochondria are evident in cells of all ages (fig. 53). The cells of the inner epidermis are of variable outline, thick-walled, and covered by a thin cuticle (fig. 1).

Cracking of rind

The cracking of the rind is the crucial stage of water-spot injury, since it allows increased penetration of water and also the entrance of mold spores (figs. 41–47). For intensive study of cracking, the tearing of the epidermis and of the underlying tissues may be induced more or less readily in maturing fruits by placing them in a rain chamber, or under running water, for a day or more. It may, however, be induced more consistently if segments of rind are placed, albedo downward, in water. The same effect is obtained by slicing off a sliver of rind from a mature fruit and standing the latter, wound downward, in a Petri dish of water. Within 1–3 hours cracks generally appear around the wound and radiate upward therefrom as the tissue becomes visibly waterlogged. The pattern of cracking induced in the peel by the above methods is similar to that seen in the field at the onset of water-spot injury.

The failure of the epidermis to keep pace with the volume increase of the underlying tissues is not peculiar to the mature fruit. In green oranges, ± 2 cm. in diameter, a relative lag in the rate of cell division and expansion may result in strain and incipient rupture of the middle lamella (figs. 41–43). At this stage of growth, however, deposition of cutin apparently is sufficiently active to prevent the development of an open wound. In fresh material stained with Sudan III, and in epidermal tissue isolated by maceration, such cutinized areas are readily observed. They may occur in any section of the epidermis, both near stomata and in the intervening areas. Occasionally, they lie immediately adjacent to one or more discolored, nonliving cells which, presumably, have been damaged by insect or other agency (fig. 42).

In mature fruits, also, similar incipient cracks, discernible only under high power or oil immersion, may be evident. As in younger fruits, cutin deposition may keep pace with underlying expansion, and further damage be prevented. If at last the epidermis is strained to the breaking-point, the initial split may occur at any point in the middle lamella, but thereafter, as the crack extends, it may rip through the center of living cells, leaving along the sides a fringe of moribund protoplasts and jagged remnants of wall.

The pattern of cracking appears on first sight entirely erratic, but, as expected, the basic plan depends on the topography of the skin—in particular, on the distribution of oil glands and stomata. As already observed, each oil gland is encircled by one or more orbits of stomata which lie along the slopes of the interglandular ridges. Conspicuous clefts appear first along the summits of the ridges, and, at the same time, furrows outline the gland margins (fig. 44). Finer cracks next arise around the accessory cells of the stomatal areas and also radiate in all directions to form a highly complex network. The depth of the cracks is variable and may be followed from the mere rupture of the in-

sufficiently elastic cuticle through the tearing of the epidermis and of the outer layers of the underlying flavedo (figs. 45–47).

If cracked fruits are left in comparatively dry air, wound healing follows in the normal way by cicatrization. Within 24 hours the entire network of a cracked surface is etched with a grayish-buff deposit (31), and a day or two later, in sections treated with Biebrich Scarlet or Sudan III, traces of cutin or suberin are apparent in the outer cell layers. Damaged cells adjacent to the cracks become shrunken and flattened, while ruptured oil glands, bereft of content, persist as minute empty cups. Suberization or cutinization is evident to a depth of one or more layers of cells. Beneath the cutinized surface, cell division is initiated in a phellogen, the cells of which are polygonal rather than cubical or rectangular as in the typical cork of the stem.

Lignification of epidermis

Sporadically, in mature fruits of all sizes, generally toward the end of the growing season, lignification may occur in small areas of the epidermis. The initial stimulus for this fundamental chemical change in cell-wall composition has not been determined. The existence of such comparatively nonextensible areas probably affects the tendency to crack in some fruits. As a symptom, however, it does not appear to be widespread.

Absorption of aqueous stains

The ready absorption of water by *Citrus* rind is doubtless a potent factor in water-spot injury (23). Absorption may be readily demonstrated by immersion of mature fruits for varying periods of time (2 hours to overnight) at different temperatures (±65° F., ±28° F.) in 0.01% aqueous methylene blue. Qualitatively, the results produced by brief and by prolonged staining are essentially similar, though accumulation of dye is naturally intensified after the longer immersion.

That the permeability of epidermal cells is variable is evident from the following preliminary observations:

1. The staining of cell vacuoles is well marked in outer accessory cells around older stomata, whereas in inner younger accessories it may be faint or absent. All accessory cells, as already noted, are thinly cutinized. Stomatal plugs, when present, are invariably dark blue in color, while the guard cells may or may not show dye absorption (figs. 54, 55).

2. The permeability of younger cells is, in general, erratic. Accessories and guard cells around younger stomata may or may not be faintly or heavily stained. In the interstomatal areas dye penetration is sporadic and evident chiefly in older cells. The age of cells is indicated by the uniformity of thickness of the radial walls, in contrast to the disparity obvious in cells which have recently divided.

3. Oil-gland cover cells of older and younger glands are consistently stained. In radial section dye penetration is evident in both gland-sheath and glandular cells.

4. Wherever visible cracks occur, the staining of epidermal and of underlying cells is immediate and intense. Minute cracks in the middle lamella, detected only microscopically, appear as discrete, deeply stained, pinpoint areas (fig. 56).

Some effects of freezing

During winter, damage by occasional frosts may not be effectively prevented by smudging. The specific susceptibility of the fruit rind cells to low temperature has been discussed in detail in a recent

paper (**28**). The effect of low temperature in the field in relation to water-spot injury is still a matter for physiological investigation. As far as the structure of the rind as a whole is concerned, the result of intense or prolonged freezing is tissue death (**5, 27**). Oil glands are ruptured, cracks appear, and water loss causes cell shrinkage. Beneath the depressed, discolored, shrunken cutinized or suberized areas of frost damage, a phellogen is initiated, the activity of which, however, appears to be limited to one or two tangential divisions only. Frost injury similar to that in the field may be induced experimentally in picked fruit by freezing localized areas of the rind.

In addition to visible surface damage, freezing results in internal tearing. Rupture occurs generally between hypodermis and flavedo and is evident both in field and in experimentally frozen fruit.

A curious phenomenon which can best be described as "sweating" occurs when fruits are first removed from a refrigerator ($\pm 28°$ F.) to warm air ($\pm 70°$ F.). Freshly picked fruits are placed in a refrigerator and dotted with drops of water which are marked by rings of India ink. In a short time the water drops freeze, but within 24 hours the ice evaporates. On removal from the refrigerator, the entire surface of the orange, when immediately and carefully examined with a hand lens, appears at first uniformly dull. Within 60 seconds, however, the waterdrop areas glisten with moisture. The liquid exudes from apparently intact epidermal or accessory cells, not from oil glands, nor through stomata, nor through microscopically observable surface cracks. The fluid is sufficiently profuse to ooze downward and collect temporarily in the depressions of the oil glands. In dry air the moisture evaporates in a short time and leaves an intact epidermal surface. This "sweating" is not to be confused with the normal condensation of moisture which occurs over the chilled fruit within the next 5 minutes after removal from the cold temperature.

The explanation of sweating is still to be sought, perhaps in the intricate structure of the cell wall. The walls of living cells are saturated with water. On freezing, differential expansion in the intermicellar spaces may result in micel dislocation and the formation of submicroscopic clefts. Through such channels in the weakened wall, on thawing which follows immediately after release to warm air, surplus fluid "sweat" escapes. The nature of the exudate is undetermined but is presumably water with a trace of natural oil.

Discussion

In regard to the orchard practice of oil-spraying, it is established that the incidence of water spot is consistently higher in sprayed than in fumigated or untreated groves (**10, 11, 12, 14, 37**). Oil is known to penetrate the orange tissues (**1, 17, 25, 32**), but no anatomical differences (e.g., hypertrophy or dwarfing of cells, meristematic activity, etc.) were observed in the unsprayed, sprayed, or even oil-dipped fruits. The presence of oil in the walls and in the protoplasts of epidermal and underlying cells may affect their physiological condition, but no significant differences in permeability were indicated in preliminary experiments on dye absorption.

Further investigation may show that specific cells of the rind, after oil-spraying, are less tolerant to low temperature than untreated cells (**28**). The presence of frost-killed cells would prove an additional factor in rind weakness, since it

would increase permeability and tendency to crack.

Apart from possible oil-spray damage, the rind of the Washington navel is structurally weak, owing to scattered areas of lightly cutinized cells in the epidermis. Chemical analyses of the fruit as a whole indicate that, during ripening, sugar and nitrogen content approach a maximum (**3, 4, 19, 36**) and that, at the same time, significant changes occur in the pectic substances of the cell walls (**16, 30, 33**). Thus both osmotic conditions and imbibition capacity of the wall favor water absorption and retention. At maturity, therefore, the balance between epidermal extension and volume increase is critical but, under normal weather conditions, is maintained. If, however, heavy rains, alternating or not with frosts, occur during this period, the physiological equilibrium may be upset, and excessive water intake and localized accumulation stretch the epidermis to the cracking-point.

Recent work indicates that all varieties of navel oranges are not equally susceptible to water spot (**21**). Some, less desirable from the commercial standpoint, are more resistant than the Washington navel. No significant anatomical differences were observed in the rind structure of susceptible and nonsusceptible varieties.

Summary

1. The symptoms of water-spot injury, as already described by other investigators, are waterlogging and subsequent cracking of the rind of the ripening Washington navel orange.

2. The epidermis of the rind of the Washington navel, as of other varieties, is structurally weak. Cell division continues during maturity, and discrete, new, thin-walled cells are consistently present. In addition, the thinly cutinized accessory cells constitute zones of weakness.

3. That the epidermis and underlying tissues are readily permeable to water is demonstrated by immersion in vital stains. Methylene blue appears in the cell vacuoles, particularly of accessory cells and gland-cover and gland-sheath cells.

4. The sponginess of the rind, especially of the albedo, facilitates water accumulation.

5. The albedo differentiates from tetrakaidekahedral meristematic cells and, when mature, consists of an open meshwork of eight-armed cells interconnected by plasmodesmata.

6. Structural weakness is already apparent in the young fruit. Incipient microscopic cracks, owing to normal growth strains or insect puncture, are, however, healed by concurrent deposition of cutin.

7. In the mature fruit the balance between epidermal extension and increase in volume of underlying tissues is critical and may be upset by excessive water absorption during winter rains. The cracks which result may heal in dry weather by normal cicatrization or, in continued rain, may remain open and allow the entrance of fungal spores.

8. Why the Washington navel orange is more susceptible to water spot than other varieties which ripen at the same time is undetermined. No significant differences in rind structure are evident.

9. The orchard practice of oil-spraying does not visibly affect rind structure. No anatomical difference is observed between untreated and oil-sprayed, or even oil-dipped, fruits.

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CITRUS LEAF STOMATA: STRUCTURE, COMPOSITION, AND PORE SIZE IN RELATION TO PENETRATION OF LIQUIDS[1]

F. M. TURRELL

Introduction

Stomatal numbers and sizes have received considerable attention in plant investigations. Either number or size, or both, have been used in theoretical considerations of transpiration (**8, 17**), diffusion rates (**3**), genetic composition (**7**), age of clones (**2**), height of leaf insertion on the stem (**15, 25**), xeromorphy (**15**), differentiation of sun- and shade-leaf development (**4**), ecological habitat (**18**), irrigation (**16**), and inorganic nutrition (**13**). In some cases stomatal pore size has been reported, but in much of the work stomatal size is reported as guard-cell size.

REED and HIRANO (**19**) and HIRANO (**9**) have made excellent and extensive stomatal counts in citrus leaves; but no estimates have been made of stomatal pore sizes in citrus leaves, though pore measurements were made by ECKERSON (**6**) for a rather sizable number of other genera.

Stomatal pore size in citrus leaves is of practical importance because penetration of sprays used for control of insect and fungus pests of citrus may be a function of pore size, shape, and conformation, as well as of spray composition. This conclusion is fortified by consideration of the well-known formula for the rise of a liquid in a capillary tube

$$h = \frac{2\gamma \cos \theta}{rg}, \qquad (1)$$

where h is the height of rise (i.e., a measure of the attractive force involved), γ is the surface tension of the liquid, θ is the contact angle, r is the radius of the tube, and g is gravitational force. These relationships were discussed by HOSKINS (**11**) and by WILLOWS and HATSCHEK (**24**).

Material and methods

Stomatal structure was studied from samples cut from fresh citrus leaves, which were killed in formalin–acetic acid–alcohol and made into permanent microscope slides by standard methods. Both transverse and tangential sections were examined with a microscope fitted with an oil-immersion objective. Photographs and drawings were made and used as a basis in making a plastic model of a stoma for interpreting the external stomatal structure.

For the studies on stomatal pore size, five average-sized sun leaves were selected from one tree each of Marsh grapefruit, Valencia orange, and Washington navel orange; ten sun leaves, five medium-sized and five small-sized, were selected from a Eureka lemon tree. All the trees were about 12 years old, in good condition, and growing in plots at the University of California Citrus Experiment Station, Riverside. The leaves were picked fresh as needed and were washed with water. The resinous stomatal plugs were removed with alternate applications of alcohol and xylene, using a cotton swab. After killing in boiling alcohol, freehand tangential sections of the lower epidermis were made, stained in ruthenium red, mounted in glycerine, and subjected to a direct microscopical examination, using a high-power (62×) dry ob-

[1] Paper no. 557, University of California Citrus Experiment Station, Riverside, Calif.

jective, in combination with a 12× ocular fitted with an ocular micrometer. The dimensions of ten or more stomatal pores were measured on each leaf. Calibrations indicated that one division of the ocular micrometer was equal to 1.18 μ. Readings could be made to one-half of a division, giving an accuracy of about 0.59 μ.

The ellipticity of the stomatal pores was determined by making enlargements (4400×) of camera lucida drawings of stomatal pores and by testing points (p) on the curve in the first quadrant by direct measurement of r and r_1 and comparison with calculated values, using the observed major (a) and minor (b) semiaxes of the pore to determine the foci (c) for measured and calculated values (fig. 1). The points (p) were determined by erecting perpendiculars to the x-axis at arbitrarily set points, $x_1 \ldots x_n$. The value c was calculated from the formula

$$c = \sqrt{a^2 - b^2},$$

while r and r_1 were calculated, respectively, from the formulas

$$r = a + \frac{cx}{a} \quad \text{and} \quad r_1 = a - \frac{cx}{a}.$$

Stomatal pore perimeter and area were calculated from the formulas for the perimeter (P) and area (A) of an ellipse

$$P = 2\pi\sqrt{\frac{a^2 + b^2}{2}} \quad \text{and} \quad A = \pi a b,$$

where a is the major semiaxis (length) and b the minor semiaxis (width).

Results

It was determined by the methods of mathematical analysis described above that stomatal pores of Washington navel orange leaves are elliptical. The observed elliptic constants, and the calculated and observed variables, are shown in table 1.

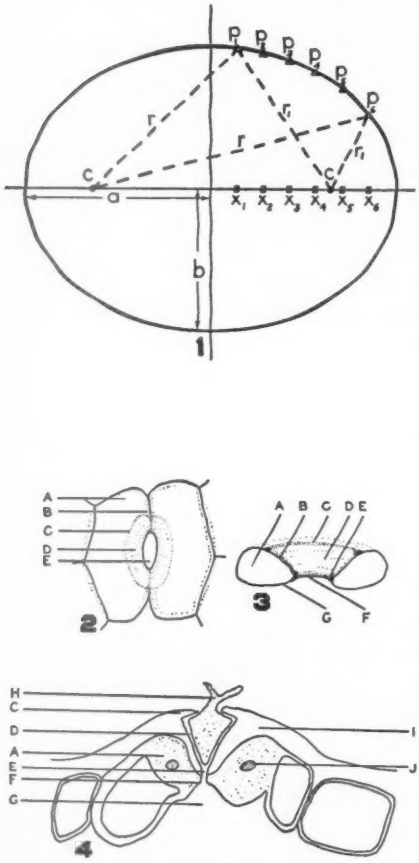
TABLE 1

COMPARISON OF CALCULATED AND OBSERVED VALUES OF r AND r_1 FOR VARIOUS VALUES OF x, FOR STOMATAL PORE I, WHEN $a = 2.05$, $b = 0.7$ (OBSERVED), AND $c = 1.93$ (CALCULATED); AND FOR STOMATAL PORE II, WHEN $a = 2.28$ AND $b = 0.62$ (OBSERVED), AND $c = 2.19$ (CALCULATED)*

p SUBSCRIPT	x	PORE I				PORE II			
		r		r_1		r		r_1	
		Calculated	Observed	Calculated	Observed	Calculated	Observed	Calculated	Observed
1	0.50	2.52	2.50	1.58	1.52	2.76	2.75	1.80	1.80
2	1.00	2.99	2.99	1.11	1.10	3.24	3.24	1.32	1.32
3	1.50	3.46	3.46	0.64	0.65	3.72	3.74	0.84	0.86
4	1.93	3.86	3.82	0.24	0.30	.....	.....	.....	.....
5	2.00	.....	.....	.....	.....	4.20	4.19	0.36	0.38

* For explanation of symbols and methods see text.

The general structure of a stoma of a Valencia orange leaf is shown in figures 2–4. Notable features are the ridges surrounding the stomatal chamber, the loosely fitting stomatal plug which nearly fills the stomatal chamber (the space between the plug and the chamber wall is about 0.5 μ), and the extremely large stomatal chamber (7.8 μ deep), at the bottom of which lies the elliptical stomatal pore. BROWN and ESCOMBE (3) found the stomatal pore ("tube") in *Helianthus* to be 14 μ in length. In the



FIGS. 1–4.—Fig. 1, diagram of an ellipse, showing method used for testing ellipticity of stomatal pore: a, major semiaxis; b, minor semiaxis; c, foci; x_1, x_2 . . . . , arbitrary values on x-axis; p_1, p_2 . . . . , points cut off on ellipse by perpendiculars erected at x_1, x_2 . . . . ; r, distance from $-c$ to p; r_1, distance from $+c$ to p. Fig. 2, diagram of surface view of stoma of Valencia orange leaf: *A*, guard cell; *B*, stomatal crevice; *C*, stomatal ridge; *D*, stomatal chamber (outer vestibular opening—*Vorhoff*); and *E*, stomatal pore. Fig. 3, diagram of longitudinal cross section of stoma of Valencia orange leaf: *F*, stomatal pore wall; *G*, substomatal chamber (inner vestibular opening—*Hinterhoff*). Fig. 4, diagram of transverse section through stoma of Valencia orange leaf, at center: *H*, stomatal plug; *I*, cuticle; and *J*, nucleus.

Valencia orange leaf, however, the walls of the stomatal pore are only 3.1 μ in length, so that even though the substomatal chamber formed by the inner side of the guard cells is shallow, the stomatal pore appears to be a perforation in a thin membrane formed by an extension or protrusion of the inner walls of the guard cells. On the outer surface the line of junction of the guard cells is somewhat sunken and forms a miniature crevice leading to the stomatal chamber. This crevice is not interrupted by the stomatal ridge lying along the edge of the stomatal chamber, so that a line of flow apparently exists from the surface to the interior of the stoma. A cuticle about 3 μ thick covers the outer surface of the guard cells, forms the stomatal ridge, and extends downward into the stomatal chamber to the entrance of the stomatal pore. Although the stomatal chamber is completely cutinized, the stomatal pore walls are not. The pore walls react strongly with ruthenium red, and this suggests that they may be constituted either entirely or in part of pectinaceous material. Examination of the pore region with a polarizing microscope showed only slight and occasional streaks of double-refractive material. This seems to substantiate the staining evidence that the walls of the stomatal pore are pectinaceous. The stomatal plug, according to other investigations (10), seems to be of resinous composition.

The average lengths (2*a*) and widths (2*b*), with standard errors, of fifty or more stomatal pores of leaves of each of the common commercial varieties of citrus are shown in table 2, in conjunction with the maximum and minimum pore lengths and widths. In the leaves of Eureka lemon the difference between pore lengths of medium and small leaves is perhaps not significant, but pore

width appears significantly smaller in the small leaves. Average stomatal pore length was smallest in the leaves of Washington navel orange, and larger in increasing order in Eureka lemon, Marsh grapefruit, and Valencia orange. Pores of small leaves of Eureka lemon and of medium-sized leaves of Marsh grapefruit, though about equal in width, were narrower than those of Washington navel orange. Leaves of Washington navel orange had the second smallest average pore width. Pores of medium-sized leaves of Eureka lemon were next larger in width, and those of Valencia orange were largest. Stomatal pore size was highly variable, Marsh grapefruit and Washington navel orange showing the least variability, Eureka lemon and Valencia orange the most.

TABLE 2

STOMATAL PORE SIZES OF CITRUS LEAVES

Kind of leaf	Number of pores measured	Leaf size	Pore length (μ)			Pore width (μ)			Average pore perimeter* (μ)	Average pore area* (μ²)
			Average	Maximum	Minimum	Average	Maximum	Minimum		
Eureka lemon...	50	Medium	7.04±1.73†	12.4	3.5	3.08±0.97†	5.9	1.2	17.1	17.0
Eureka lemon...	50	Small	7.13	12.4	2.4	2.07	5.9	1.2	16.5	11.6
Marsh grapefruit	50	Medium	8.06±1.59	12.0	4.7	2.08±0.50	3.5	1.2	18.5	13.2
Valencia orange.	70	Medium	8.91±3.10	14.2	5.3	3.80±0.98	6.5	1.8	21.5	26.6
Washington navel orange....	50	Medium	4.78±0.86	8.3	3.5	2.32±0.82	4.7	1.2	11.8	8.7

* Considering the stomatal pore as an ellipse. † Standard error.

The average pore perimeters, in order, from smallest to largest, were those of Washington navel orange, of small leaves of Eureka lemon, medium-sized leaves of Eureka lemon, Marsh grapefruit, and Valencia orange (table 2). For pore areas the order of size was the same, except that pore areas of Marsh grapefruit were smaller than those of medium leaves of Eureka lemon.

Discussion

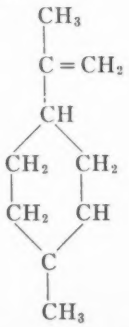
The term "stoma" is derived directly from the Greek στομα, meaning "mouth." JACKSON (12) defines it as "a breathing pore or aperture in the epidermis, surrounded by two guard-cells, leading into an intercellular space communicating with internal tissue." From this definition it may be inferred that "stoma" applies to the pore between two adjacent guard cells. Generally, however, as indicated in papers already cited, the guard cells have been included as part of the stoma. The nomenclature used in the present paper has, so far as possible, been based on common usage in the literature. Thus "stoma" has been employed in the general sense of including a stomatal pore and two guard cells. In a special sense, the stomatal pore has been limited in this paper to mean the connecting space between the stomatal chamber and the substomatal chamber. The terms "stomatal ridge," "stomatal crevice," and "stomatal plug" have been employed without precedent in order to facilitate discussion of stomatal structure in relation to spray penetration.

The structure of the citrus stoma is such that *a liquid of the proper charac-*

teristics might, if placed on the surface of the leaf, be drawn rapidly into the mesophyll. Liquid accumulating in the narrow stomatal crevice, through action of the various vectors of gravitational force operating when the leaf is moved by air currents, could be drawn to the edge of the stomatal plug, where it would be drawn rapidly under by capillary force, even though the stomata are confined to the lower leaf surface (except over the major veins) where entrance is hindered by the action of gravitational force. Inasmuch as the distance between the plug and the stomatal chamber wall is about half a micron, the capillary force might be sizable. Factors that would be expected to influence capillary flow, other than pore size, are the pectic nature of the constituents of the stomatal tube wall (**21**), the elliptical pore of small width (averaging 2–4 μ), the resinous stomatal plug, and the cuticular covering of the stomatal chamber. Rate of flow for a particular liquid would, of course, be influenced by the magnitude of the molecular forces involved in the liquid-solid interfaces, as well as by the size of the free space in the channel between the stomatal plug and chamber wall, and by the pore area.

In the monsoon region of forests of China and the Malay Archipelago (**22**), the problem of gas diffusion into and out of the citrus leaf mesophyll could be critical during periods of intense insolation immediately following heavy rains, if the stomata and intercellular spaces of the mesophyll became filled with water. The likelihood of the latter occurrence is probably small, however, as the structure of the citrus stoma seems well adapted to the native habitat of the cultivated species of *Citrus*. Resinous stomatal plugs and cutinized stomatal chambers perhaps effectively prevent the stomata, and consequently the intercellular spaces, from filling with water.

The operation of the mechanism in preventing water penetration may be explained as follows: The contact angle (θ) between distilled water and waxed capillary glass tubes, as has been shown by EBELING (**5**), is large (i.e., cos θ is small), and the height of rise of water in the tubes is zero; whereas in clean unwaxed capillary glass tubes the contact angle θ is small, and the height of rise is more than 2 cm. This shows that, where contact angles are large, surface forces are small. Inasmuch as the citrus resin (of which the citrus stomatal plug consists) is probably formed by polymerization and oxidation (of low order) of the ethereal oils, as are most resins, one might expect a citrus resin to be polymerized from a common ethereal oil such as limonene, which is found in citrus. The structural formula indicates that this substance is relatively nonpolar and that the contact angle (θ) of water with it would be large.



Surface forces are known to be the result, in part at least, of the structures of the molecules making up the surface. The 1,8(9)-p-menthadiene (limonene), though not so symmetrical as the parent molecule menthane, electrically, is fairly symmetrical. There are no O-H, N-H, S-H, or halogen bond moments to con-

tribute large charges. Such charges as would effect polarity arise primarily around the *1* and *4* carbons of the ring and from C-H bonds. Rough calculations of the bond moments by differences of electro-negativities (**23**) indicate a moment of about 1.6 Debye units, which is relatively small and indicates that the water contact angle with limonene and its polymers would be large.

MARKLEY *et al.* (**14**) have isolated and identified some of the constituents of grapefruit peel cuticle and found solid fatty acids having a mean molecular weight corresponding to $C_{32}H_{64}O_2$; the unsaturated fatty acids linolenic ($C_{17}H_{29}COOH$), linoleic ($C_{17}H_{31}COOH$), and oleic ($C_{17}H_{33}COOH$); a sapogenic ketone, $C_{30}H_{52}CO$; hydrocarbons $C_{29}H_{60}$ and $C_{31}H_{64}$; a phytosterol, $C_{28}H_{47}OH$; and umbelliferone, $C_9H_6O_3$. These compounds, likewise, obviously have a very small degree of polarity (as the number of OH and CO groups are few), and the contact angle (θ) of water with them therefore must be large. EBELING (**5**) has investigated the contact angles of water on the intact ventral surfaces of various citrus leaves and found them to be high (orange, 69°54′; grapefruit, 67°21′; lemon, 76°42′). The fact that there is such a small space (0.5 μ or less) between the stomatal plug and the stomatal chamber wall suggests that strong capillary forces might be active (as may be seen by making r very small in eq. [1]). However, when the contact angle is large, cos θ is small; this indicates that surface forces will be small (as may be seen by making cos θ small in eq. [1]) and that any significant penetration of citrus stomata by water is unlikely. In fact, TURRELL and KLOTZ (**21**) found that, although large and small oranges have statistically significant differences in the number of stomata in the peel, there were no significant differences in the amount of water absorbed when the fruits were immersed.

It seems, therefore, that the above explanation serves to show why the water-spotting of Washington navel orange fruit is a result of water penetration of the peel through cracks and abrasions and not through stomata. With oil sprays, the contact angles (**5**) may be moderately low ($\theta = 32°5'$) to extremely low ($\theta = 11°26'$), and penetration of the stomata may be expected. With water sprays containing certain spreading and wetting agents, contact angles in tests on *Viburnum* leaves (**5**) were moderately low (45°22′) to fairly low (27°57′). There is some evidence that, so far as oil sprays are concerned, penetration of citrus stomata has occurred. ROHRBAUGH (**20**) observed that, when oil was painted on the under surfaces of orange leaves, it penetrated into the mesophyll more rapidly than when painted on the upper surfaces, owing to entrance through the stomata, which are present only on the underside of the blade.

Penetration of the leaf by zinc, iron, manganese, and other salts carried in emulsions in oil sprays, or in water sprays containing certain wetting or spreading agents, may be expected to enter the stomata rather readily. Certain spreading or wetting agents may be more effective than others, however. EBELING (**5**) has shown that the contact angle (θ) varies with the wetting agent and with the kind of waxy substrate.

In view of the peculiar structure of the plugged citrus stoma, questions naturally arise: Are the stomata operative? If they are, how much are pore dimensions affected? Transpiration measurements by BARTHOLOMEW (**1**) showed a light-to-shade decrease in dorsal/ventral transpiration ratio from 86 mg./752 mg. to

52 mg./227 mg. of water lost. On the reversed sequence (shade to light), the ratios were 43 mg./252 mg. to 69 mg./684 mg. This strongly suggests that the stomata are operative, since the effects of temperature were clearly largely eliminated by the change in sequence.

In view of the heavy cuticular layer over the guard cells in the region of the stomatal chamber (fig. 4), it seems unlikely that the chamber dimensions would vary greatly. The stomatal plug so nearly fills the stomatal chamber that closure of more than 1 or 2 μ of this portion of the stomata would be impossible. However, this closure, though involving only a small movement, might for all practical purposes stop gaseous exchange.

Examination of figure 4 indicates that the closure of the stomatal pore might easily be effected by the swelling of the guard cells, for the guard-cell walls are quite thin in the vicinity of the pore and quite thick elsewhere. We therefore may expect some variation in pore size with time of day. The leaves used in the present study were picked generally between 9:00 and 10:00 A.M. and not later than 2:00 P.M. Correlation of stomatal pore size in citrus with time of day remains to be studied.

Analysis of variance for the significance of the differences among stomatal pore lengths and pore widths for the various kinds of citrus leaves is shown in table 3. Computation of the F values for pore lengths from this table gave 46.7. Computation of the F values for pore widths for the various kinds of citrus gave 275.15. Thus, both pore lengths and pore widths are significantly different at the 1% level for the different kinds of citrus leaves.

TABLE 3

ANALYSIS OF VARIANCE OF STOMATAL PORE LENGTH AND WIDTH IN VARIOUS KINDS OF CITRUS LEAVES

Source of variation	Degrees of freedom	Sum of squares		Mean square	
		Pore length	Pore width	Pore length	Pore width
Total	219	971.7	1803	4.44	8.23
Between means of kinds	3	381.0	1428	127.00	476.00
Within means of kinds	217	590.7	375	2.72	1.73

Summary

1. The stomatal pore in Washington navel orange leaves is elliptical in shape. The average stomatal pore lengths and widths for medium-sized leaves of Eureka lemon were 7.04 × 3.08 μ; of Marsh grapefruit, 8.06 × 2.08 μ; of Valencia orange, 8.91 × 3.80 μ; and of Washington navel orange, 4.78 × 2.32 μ. Stomatal pore length and width were both highly significantly different for each kind of leaf.

2. Study of a stoma of a lemon leaf shows that it has a complicated surface structure which makes calculation of the action of surface forces on spray liquids more difficult than if based on stomatal pore size alone. Evidence based on molecular forces indicates that citrus stomata are not penetrated by water but may be penetrated by oil.

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PIGMENT GLANDS OF COTTONSEED. II. NATURE AND PROPERTIES OF GLAND WALLS

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Introduction

Gossypol, the principal pigment of cottonseed, is a hexaphenolic, dicarbonyldinaphthalene derivative (**1, 8, 22**) which cannot be assigned to any of the more important classes of plant pigments such as porphyrins, carotenoids, flavones, anthocyanins, or quinones (**15, 18**). It has been stated to be peculiar to seed of the genus *Gossypium* and a few other closely related genera (**26, 31**). The recently discovered purple- and orange-colored cottonseed pigments, gossypurpurin (**2**) and gossyfulvin (**4**), are, likewise, polyphenolic and are derivatives of gossypol.

Cottonseed pigments are unique not only in their chemical nature but also in their manner of occurrence in the seed. In summarizing the distribution of the more important classes of plant pigments, HAAS and HILL (**10**) noted that they usually occur in one of three ways: in solution in the cell sap, as in the case of most anthocyanins; as powdery deposits in the bark or on the leaves, stems, flowers, and seed capsules, as in the case of many flavones; or in the plastids, as in the case of the carotenoids and chlorophylls. Most of the pigments of cottonseed are contained in distinct morphological structures, which are relatively large ovoid or spherical bodies, 100–400 μ on the long axis, and have been variously designated as "secretion cavities," "resin glands," "oil glands," or "gossypol glands" (**31**). Pigment glands are characteristic of all species of *Gossypium* and seem to be peculiar to this genus and to a very few closely related genera of the subfamily Hibisceae (**26, 31**).

The earliest investigators of the structure of the cottonseed (**6, 12, 13**) noted the occurrence of pigment glands and reported the existence of a gland "membrane" made up of two layers, the outer layer consisting of thin-walled, tangentially flattened cells and the inner layer of mucilaginized material in which traces of cell walls were still evident. HANAUSEK (**12, 13**) noted that successive treatment of the gland with water and solutions of hydrochloric acid and potassium hydroxide revealed the mucilaginized layer as a yellow, folded, and laminated mass. Subsequent investigators have neither added to the early descriptions of the gland "membrane" nor confirmed its existence.

The pigment glands are very resistant to mechanical rupture as well as to the action of a number of organic liquids; and, when cottonseed kernels are comminuted while in contact with a mixture of inert organic solvents (density 1.378 gm./ml.), the intact glands will rise to the surface, with the other seed tissue falling to the bottom (**5**). Although the assumption that the pigments are confined within a resistant membrane seemed to constitute the most logical explanation of their behavior, it might be argued that they occur in peculiarly resistant agglomerations held together by surface forces, as has been suggested in the case of plant plastids (**32**).

The present investigation was undertaken with the object of obtaining further information concerning the structure and properties of the pigment glands. All experiments were carried out with samples of three pure-bred strains of *Gossypium hirsutum*.

Observations

Pigment glands are profusely distributed over the cotyledons and hypocotyls and are absent only from the lower part of the radicle of the cottonseed embryo. Their relatively large size, as well as their distribution, is shown in figure 1, which represents a free-hand section of a seed.

Microscopic examination confirmed the observations of HANAUSEK (13) and VON BRETFELD (6) that contact with water induces immediate rupture of the glands. Rupture occurs within a fraction of a second after immersion, and the contents flow out in vigorously moving streams of minutely divided, suspended particles in lively motion. After the gland is emptied, a nearly transparent structure remains which retains the shape of the original gland. This series of changes is shown in figure 2. In some cases the "streaming" is spasmodic, at first very vigorous, slowing down after a short time, and later renewing its original intensity. In a few cases as many as four simultaneous streams of suspended material flowed from a single gland.

The reaction of separated glands was the same regardless of the means employed for their separation—that is, whether separated manually or by the flotation process. Glands allowed to remain in contact with the surrounding tissue and oil on the surfaces of freshly cut cross sections of cottonseed reacted with water in a fashion entirely analogous to that of separated glands. Preliminary defatting of ground or flaked cottonseed did not alter the reaction of intact glands with water.

PIGMENT GLAND WALL

STRUCTURE.—In order to investigate the structure of the emptied gland, individual glands were soaked overnight in alcoholic acetic acid (5% acetic acid in 95% ethyl alcohol) and then in 25% aqueous ammonium hydroxide. They were then mounted in water for microscopic examination. This treatment freed the glands of their contents and removed particles adhering to the outside. Gland walls so prepared are observed to be composed of five to eight, relatively thick and almost colorless, curved plates of irregular shape, fitted together in a pattern somewhat resembling the cover of a baseball (fig. 3). An unusual property is manifested in the marked tendency of the plates of the emptied glands to cohere in their original configuration even after the prolonged treatment described above.

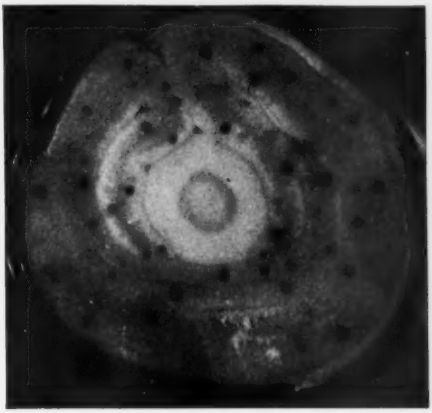

FIG. 1.—Cross section of cottonseed through radicle and cotyledons, showing distribution of pigment glands. ×14. (Photomicrograph by T. L. W. BAILEY.)

The plate-like structure of the walls is characteristic not only of separated glands but also of glands in the seed. The curved and bulging outlines of the plates may be readily observed in glands in seed sections by changing the focus of the microscope. In the case of glands separated by flotation and subsequently sieved in a dry state to remove particles of defatted meal, the outlines of the plates are particularly evident because of the packing of finely divided defatted

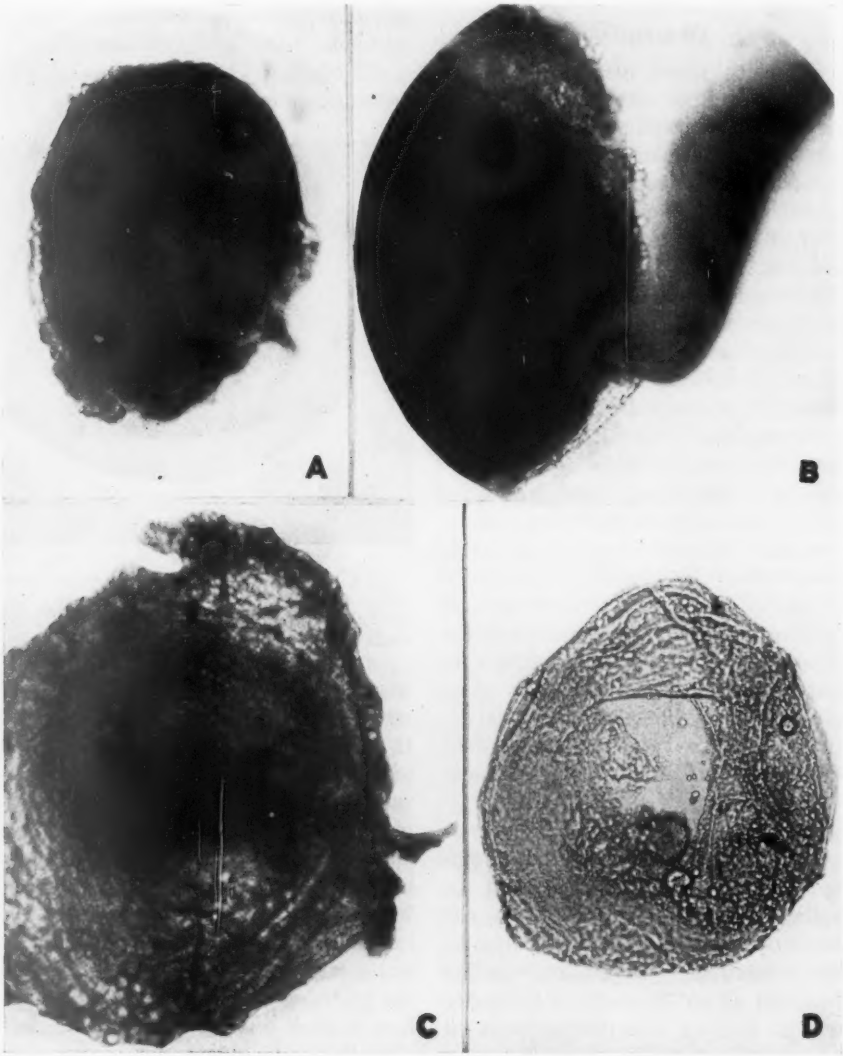

FIG. 2.—Single cottonseed pigment gland reacting with water: *A*, beginning of rupture; *B*, contents emptying; *C*, almost emptied; *D*, completely emptied. ×500. (Photomicrographs by M. L. ROLLINS.)

meal particles along the seams between the plates.

The action of water alone is entirely analogous to that of aqueous reagents. Figure 2*D* shows that, despite the vigorous reaction involved in the discharge of the pigments during immersion in water, only the front plate of the gland has been removed, and the other plates have remained joined together as in the original intact gland.

observed in the ruptured glands. Portions of gland walls consisting of several adjoined plates have the appearance of empty shells floating in the liquid of the mounting medium, and crushing them reveals no inner contents. Closer observation of what at first appears to be a circular structure in the center of each plate reveals it to be merely a thickened area which probably accounts for the curvature of the plates. The striations

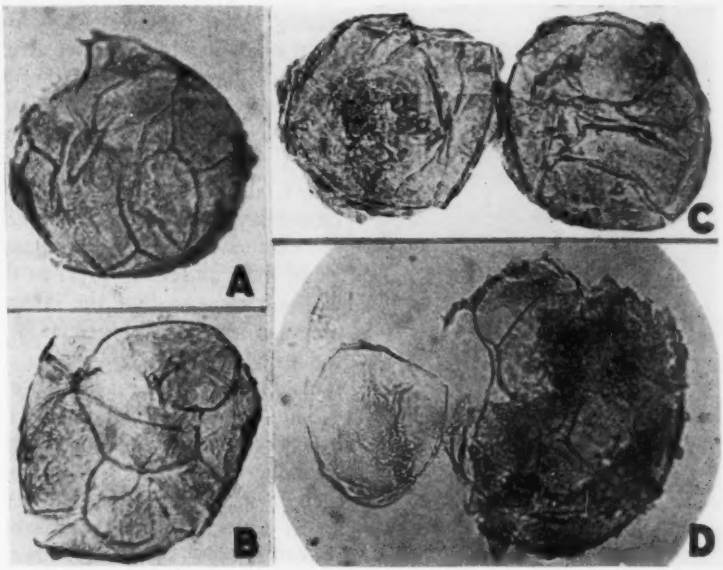

FIG. 3.—Emptied cottonseed pigment glands showing wall structure. ×250. (Photomicrographs by M. L. ROLLINS.)

On the basis of striations observed in sections of cottonseed pigment glands, previous investigators (31) have reported the existence of residual cell walls in the interior. The mechanical strength of the glands and the tendency of the plates to remain in position after the glands are emptied, as well as the spasmodic nature of the emptying process, also suggested the existence of secondary or internal walls. During the present investigation, however, no internal structure could be reported by STANFORD and VIEHOEVER (31) are readily observed in sections of the glands (fig. 4). However, since examination of a large number of cross sections reveals no regular arrangement of the striations, it can be concluded that they are merely fissures produced by mechanical pressure on the gelatinous contents of the glands.

CHEMICAL COMPOSITION.—Microchemical investigation of the nature of cleaned and emptied glands yielded the results

reported in table 1. Conclusions concerning the chemical nature of the constituents of the walls were based on the properties described by HAAS and HILL (10).

The absence of proteins was indicated by the failure of the emptied gland to react with Millon's reagent; the absence of lignin and pentosans, by lack of reaction with phloroglucin and hydrochloric acid.[1]

Staining of the outer surface of water-washed gland walls with Sudan IV indicated a coating which might be suberin, cutin, or wax. Since the reaction with Sudan IV was not affected by prior washing of the glands with petroleum naphtha, it was concluded that little, if any, wax occurred in the coating. A further indication of the presence of suberin and cutin was the yellow stain obtained with concentrated potassium hydroxide. A negative test with iodine–zinc chloride after heating showed that the compound was cutin rather than suberin.

FIG. 4.—Cross section of cottonseed pigment gland showing striations. ×440. (Photomicrograph by T. L. W. BAILEY and I. V. DEGRUY.)

Emptied glands stained with Sudan IV were mottled in appearance. Observation of the distribution of Sudan IV applied to sections of emptied glands in seed sections showed that cutin occurs as an uneven, continuous, outer coating of the glands. After the presence of cutin had been established by staining reactions, it was possible to obtain further information concerning its distribution on the gland walls. Microscopic examination of intact glands, without application of stains, showed a relatively thick transparent layer of material in the region of the centers of the plates composing the gland walls and a thin layer at the edges of the plates, so that the intact glands are very irregular in outline. In view of the previously described staining reactions, this thick layer is probably cutin.

The cutin was completely removed from glands which had been immersed in alcoholic acetic acid for 24 hours and then in aqueous ammonium hydroxide for 24 hours. Thus, as with cutin in the skin of apples (23), it appears probable that the cutin is not chemically combined with the other wall constituents but forms a separate layer or incrustation around the glands.

The positive reactions obtained with diagnostic cellulose stains indicated that cellulose is the principal wall constituent, but the modified color observed suggested the presence of other substances. Treatment of the walls with ammoniacal cupric hydroxide resulted in their partial solution. However, the residual material retained the original configuration so that it had the appearance of "ghosts" of

[1] Recent microchemical observations of the reaction of cottonseed with phloroglucin-hydrochloric acid and other pentosan reagents (28, 29, 31) showed the reaction to be localized in the pigment glands. The present observation that pentosans or pentosan-like substances do not occur in the walls confirms the further observation (9) that these compounds constitute part of the gland contents.

the plates composing the walls. Consequently, it was concluded that the cellulose is impregnated with the materials responsible for the modified colors obtained with cellulose stains.

Efforts to determine the nature of the material with which the cellulose was impregnated were not conclusive. The original water-washed glands, as well as those treated with cupric ammonium hydroxide, developed a red-purple color with ruthenium red which suggested the presence of pectins, hemicelluloses (14), or other uronic acid derivatives. Boiling the walls in 0.5% ammonium oxalate to remove pectins left "ghosts" which still stained with ruthenium red. After emptied glands had been treated with dilute sodium hydroxide, in which hemicelluloses are soluble, the residual "ghosts" likewise gave a positive reaction with ruthenium red. Moreover, following successive treatments with dilute aqueous hydrochloric acid, ammonium hydroxide, and sodium hydroxide, to remove both pectin and hemicellulose, the "ghosts" of the plates still stained with ruthenium red. Thus it appears that very stable and insoluble uronic acid derivatives other than pectin and hemicellulose form part of the gland walls. The failure of the emptied glands to react with phloroglucin and hydrochloric acid demonstrates the unusual stability in the gland walls of the materials which stain with ruthenium red.

TABLE 1

MICROCHEMICAL REACTIONS OF PIGMENT GLAND WALL

Preparation of glands*	Reagent	Observation	Conclusions
.....	Millon's reagent	No reaction	Protein absent
.....	Phloroglucin and hydrochloric acid	No reaction	Lignin and pentosan absent
.....	Sudan IV	Mottled red stain	Wax, cutin, or suberin present
Hexane washed before emptying.....	Sudan IV	Mottled red stain	Cutin or suberin present
.....	Conc. potassium hydroxide	Yellow stain	Cutin or suberin present
.....	Conc. potassium hydroxide; heat	Yellow stain	Cutin or suberin present
Heated with conc. potassium hydroxide.....	Iodine–zinc chloride	Yellow stain	Cutin present
	Sudan IV	No stain	Cutin removed
	Delafield's haematoxylin	Purple stain	Cellulose present
	Iodine green	Dark gray-green stain	Impregnated cellulose present
Alcoholic acetic acid; aqueous ammonium hydroxide.....	Iodine-potassium iodide	Yellow stain	Impregnated cellulose present
	Iodine–zinc chloride	Yellow stain	Impregnated cellulose present
	Congo red	Yellow-orange stain	Impregnated cellulose present
	Ammoniacal cupric hydroxide	Partial solution	Cellulose and hemicellulose dissolved
Ammoniacal cupric hydroxide.....	Ruthenium red	Red stain	Uronic acid derivatives present
.....	0.5% ammonium oxalate; heated	Partial solution	Pectin removed
Boiled in ammonium oxalate.....	Ruthenium red	Red stain	Uronic acid derivatives present
	Iodine–zinc chloride	Yellow stain	Impregnated cellulose
.....	Dilute sodium hydroxide	Partial solution	Hemicellulose present
Dilute sodium hydroxide.	Ruthenium red	Red stain	Uronic acid derivatives present
	Iodine–zinc chloride	Yellow stain	Impregnated cellulose

* The glands were first treated with water to empty them of pigments.

Behavior of pigment glands toward various liquids

In a previous investigation (5) microscopic observations of the effect of a number of organic liquids on cottonseed pigment glands had shown the liquids to fall into two categories with respect to their activity toward the glands: those which had little or no effect even after 24 hours of contact and those which caused the glands to lose their contents after contact for 24 hours or less. The "inert" group included hydrocarbons, such as *n*-hexane, mineral oil, benzene, and tetralin; chlorinated hydrocarbons such as carbon tetrachloride, 1,1,2-trichloroethane, 1,1,2-trichlorethylene, 1,1,2,2-tetrachloroethane, and 1,1,2,2-tetrachlorethylene; and triglycerides such as cottonseed oil. The "active" group included polar liquids of low molecular weight, such as acetone, methyl ethyl ketone, 1,4-dioxane, diethyl ether, and chloroform.

When mixtures of "inert" liquids were used for the mechanical separation of glands from other cottonseed tissue by the flotation process (5), the coloring observed in the liquid mixtures indicated some rupture of the glands. It was found that the number of glands ruptured could be greatly reduced by shortening the time of exposure to the liquids and by carrying out the separation at low temperatures. With the establishment of the existence and properties of the pigment gland wall, it seemed probable that further investigation might reveal the cause of the differences in the reaction of the glands toward various liquids.

Closer observation of the effect of water on glands revealed that the reaction occurs in several successive stages. Immediately following contact with water the contents of the gland, which are originally perfectly clear, become cloudy and lighter in color. Immediately thereafter the pigments are expelled through one or more fissures between the plates in rapidly moving streams of finely divided, suspended particles exhibiting lively Brownian movement.

In view of the insolubility of the pigments in water, the preliminary clouding of the gland contents demonstrates that water enters the glands prior to the expulsion of its contents.

The explosiveness with which the glands empty suggests that rupture might occur as the result of pressure built up by the diffusion of water into the glands through the walls. However, the effects of various saturated aqueous solutions on the glands does not support this conclusion. The glands burst almost instantly upon contact with saturated aqueous solutions of dextrin, sucrose, glucose, urea, sodium chloride, ammonium sulfate, sodium acetate, and calcium chloride. Modification of the pH of the solutions by the addition of acid or base did not affect the speed with which the glands ruptured. They burst rapidly upon immersion in glycerine containing 10% water. In pure glycerine they were not immediately affected, but each gland burst at several points after prolonged contact, and sluggishly moving streams of suspended material emanated from the surface of the gland.

Microscopic observation of the behavior of the glands in various water-miscible anhydrous organic liquids revealed that all these liquids extract the pigments from the glands but that the reaction is very much slower than with water (table 2). After contact with anhydrous methanol, ethanol, acetone, methyl ethyl ketone, or 1,4-dioxane for an hour, the glands were completely emptied. The mechanism of the reaction could not be observed because of the solubility of the

pigments in these solvents. Isopropanol had very little effect on the glands even after prolonged contact. As shown in table 2, the glands rupture more rapidly upon contact with mixtures of these organic liquids and water, and the speed of rupture increases in direct proportion to the amount of water contained in the mixture. It can be concluded that the gland walls are somewhat susceptible to the action of these polar organic molecules of low molecular weight but are much more susceptible to the action of water.

In view of these observations, it seemed probable that organic liquids, such as chloroform and diethyl ether, were themselves incapable of affecting the pigment glands; that their previously observed action resulted from the small amounts of water dissolved in them; and, furthermore, that the slowness with which these liquids extract the pigments from the glands can be attributed to their

TABLE 2

EFFECT OF SOLVENTS ON COTTONSEED PIGMENT GLANDS

Solvent	Water content (%)	Observed reaction of glands
Methanol	0	None ruptured; yellow extract darkening upon longer contact
	5	Small fraction ruptured after 15 minutes; very short purple streams* and yellow extract subsequently darkening
	10	Small fraction ruptured after 10 minutes; very slowly moving streams
	25	Most ruptured after 5 minutes; slowly moving streams
	50	Almost all ruptured immediately; rapidly moving streams
Ethanol	0	None ruptured; yellow extract darkening upon longer contact
	5	Small fraction ruptured after 20 minutes; pale-yellow extract subsequently darkening
	10	Small fraction ruptured after 10 minutes; pale-yellow extract subsequently darkening
	20	Most ruptured after 2–3 minutes; very short sluggish streams; yellow extract subsequently darkening
	50	Most immediately ruptured; rapidly moving streams; yellow precipitate
Acetone	0	Most ruptured after 10 minutes; yellow extract darkening upon longer contact
	5	Most ruptured after 3–4 minutes; yellow extract subsequently darkening
	10	Most ruptured after 2–3 minutes; yellow extract with purple precipitate
	25	Most ruptured after 1–2 minutes; yellow extract with purple precipitate
	50	Most ruptured immediately; rapidly moving streams
Dioxane	0	None ruptured; pale-yellow extract darkening upon longer contact
	10	Most ruptured after 4–5 minutes; pale-yellow extract subsequently darkening
	25	Most ruptured after 2–3 minutes; very few streams
	50	Most ruptured almost immediately; rapidly moving streams
Isopropanol	0	None ruptured; pale-yellow extract darkening upon longer contact
	5	Very small fraction ruptured after 30 minutes; yellow extract subsequently darkening
	10	Small fraction ruptured after 8 minutes; yellow extract subsequently darkening
	20	Most ruptured after 4 minutes; yellow extract subsequently darkening
	50	Most ruptured after 2–3 minutes; yellow extract subsequently darkening
	60	Most ruptured immediately; rapidly moving streams

* Streams designate finely divided suspended particles in lively motion emanating from pigment glands.

slight water content. In confirmation of this hypothesis, it was found that, whereas undried ether or chloroform extracts all the pigments[2] from the glands after contact for 24 hours (3), under the same conditions ether or chloroform, previously dried over calcium chloride or sodium sulfate, extracts only a small fraction of the pigments from either dried, separated glands or dried, finely ground cottonseed. On the other hand, when dried glands are first macerated with sharp sand so that the walls are broken, the pigment contents are instantly soluble in either dry ether or dry chloroform.

The action of commercial-grade organic liquids, such as ether or chloroform containing traces of moisture, may be postulated as follows: the water, because of its low concentration in these solvents, ruptures the gland walls very slowly; the organic liquids enter the glands through the ruptures and dissolve their contents; and the pigment solutions then slowly diffuse out of the glands through the ruptured walls. It was observed, in support of this view, that when glands were immersed in commercial-grade chloroform and the chloroform was allowed to evaporate after several hours contact, many of the glands were still intact, but some were ruptured, with mixed crystals of the pigments emanating from the interiors through the ruptures in the walls.

Formation and probable function of pigment glands

It has been reported that the first sign of the presence of pigment glands can be detected in the 15-day-old embryo, and by the 16th day the glands are as fully developed structurally as in the mature seed (27, 31). Prior to the formation of the pigment gland, a well-defined circle of cells marking the boundary of the developing gland can be noted. These cells have been assumed to constitute an integral part of the gland because of their persistence in the mature seed (13, 27, 29, 31). It is evident that this cannot be true in view of the fact that intact glands separated from the seed are free of these cells. On the other hand, the formation of the water-sensitive gland wall in the moist surroundings of the developing seed might logically be attributed to the protective action of the encircling layer of cells, which always forms before the pigment gland develops and probably functions as a protective sheath (11).

Gossypol cannot be detected in the embryo until the 15th–18th days (7, 27). After its first appearance, its concentration increases many fold and at a rate greater than that of any of the other components of the seed (7). The sequence of gland formation followed by synthesis of gossypol suggests that this very unstable polyphenolic pigment cannot be formed until it is furnished with a protected region for its development and that the subsequent rapid formation of gossypol within the gland is the result of the protective action of the gland walls.

It has been reported that the pigments of dry seed undergo relatively little change during storage (25), whereas seed of high moisture content store less well than dry seed (16, 21, 30) and yield more deeply colored oils (19, 20). It seems probable that many of the transformations occurring during the storage of moist seed can be attributed to the inadequate protective action of the sheath

[2] The gossypol content of the extracts was determined by means of the gossypol-antimony trichloride spectrophotometric method (3); and their gossypurpurin content was estimated on the basis of the height of the absorption band at 570 A characteristic of gossypurpurin (2).

of cells surrounding the pigment glands. In the presence of excessive amounts of moisture, for example, when a thick seed section is treated with several drops of water, the gland contents gradually become cloudy, indicating the entrance of moisture through the surrounding tissue. Although no clouding can be observed in the glands of seed which have been stored at such high moisture levels as to exhibit significant color deterioration in the oil, it seems probable that moisture has entered the glands in amount sufficient to affect their contents. Furthermore, the observation that increased moisture accelerates the respiration rate of stored cottonseed (**17, 20**) suggests that the polyphenolic gland pigments, like other naturally occurring phenols (**24**), participate as respiratory intermediates in the metabolism of the seed. On the basis of these combined observations it seems probable that the deeper color of the oils obtained from seed stored at higher than at optimum moisture levels can be attributed to the more active participation of the gland pigments in the respiration of the seed, and their conversion thereby into deeply colored quinoid oxidation products. These reactions would produce an increased intensity of respiration and would result in increased color in the oil.

Summary

1. The cottonseed pigment gland has been shown to possess a rigid, thick wall inclosing a gelatinous suspension of the gland pigments.

2. The gland wall consists of five to eight irregularly shaped, curved plates, which appear to be composed of cellulose impregnated with pectin, hemicellulose, and other unidentified uronic acid derivatives. The glands are heavily incrusted with cutin.

3. The pigment glands have been shown to be resistant to the action of most reagents except water and polar organic liquids of low molecular weight. Their reaction to water consists in the immediate rupture of the wall along one or more of the seams between the plates, followed by expulsion of the gland contents as finely divided suspended particles. Water-miscible, polar organic liquids of low molecular weight affect the glands very much more slowly than water; and the extremely slow action of other organic liquids has been shown to be attributable to their slight moisture content.

4. The existence of a highly resistant pigment gland wall has been proposed to explain the anomaly of the relative stability of reactive polyphenolic pigments in cottonseed. The sensitivity of the glands to moisture has been proposed to explain various changes observed during maturation of cottonseed as well as changes which occur during the storage of moist cottonseed.

The authors wish to express their appreciation of the generosity of W. R. PADEN and J. WINSTON NEELY in supplying the samples of cottonseed with which this investigation was carried out.

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PHOTOMICROGRAPHIC COMPARISON OF MILKWEED BAST AND COTTON LINT IN RELATION TO CELL-WALL STRUCTURE OF OTHER NATURAL CELLULOSE FIBERS

MARY L. ROLLINS[1]

Introduction

In competition with cotton in various phases of commercial utilization are the bast fibers of flax (*Linum usitatissimum*), hemp (*Cannabis sativa*), jute (*Chorchorus capsularis*), and ramie (*Boehmeria nivea*). Numerous other bast fibers of minor commercial importance rose to prominence during the wartime fiber scarcity, and some new to commerce were proposed as substitute fibers. Among the latter the giant milkweed (*Asclepias syriaca* [15, 26]), said to have been used as cordage fiber by various primitive peoples, was suggested as suitable for exploitation.

In the course of preliminary microscopical investigations to determine the possibilities of the bast fiber of milkweed, the incidental observation was made of a surprisingly close parallel structure between the seed hair of cotton and the bast fiber of the milkweed stem. As a consequence, the present investigation was undertaken to illustrate the morphological similarity between this bast fiber and cotton as a basic principle of nature in the structural design of cell walls of the fibrous type.

Accordingly, the two widely different types of fibers were compared under the microscope and copiously photographed. To facilitate the study of cell-wall structure, specific swelling techniques, based on the solvation of cellulose in certain reagents, were applied. The milkweed fiber, having the advantage of being relatively large, with few cell-wall layers, lends itself particularly well to microscopic study and photomicrography of cell-wall details; and photomicrographs of this fiber are of considerable aid in the interpretation of the finer and more intricate structure of the seed hair of cotton which is less easily seen.

Admittedly, none of the pictures represents the true condition of fiber cell walls in the living plant, since the chemical treatments necessary to induce sufficient swelling to reveal the inner fiber structure of the dead and desiccated cells undoubtedly alter their natural arrangement. That the fiber elements revealed in this investigation are not all artifacts, however, is supported by ample evidence scattered through the literature of a similar pattern of cell-wall structure existing in other cells of the fiber type, especially those that have gained commercial importance because of their natural strength.

Review of literature

The literature of fiber structure is voluminous, covering at least a hundred years of microscopical study. Extensive reviews of the early work were given by VAN ITERSON (34, 35), and a summary of the more recent investigations is found in chapter iii, C, of *Cellulose and Cellulose Derivatives* (29). This present paper is concerned chiefly with the review of the photographic record.

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STRUCTURE OF COTTON FIBER

The cotton fiber is a single-celled seed hair, growing out from an epidermal cell of the seed coat. Also arising from the seed coat, about 6 days after the true lint fibers have emerged, are short coarse fibers known as linters or fuzz fibers. These differ from the true lint fibers in that they vary in color, whereas the true lint is generally white. In addition, they are usually coarse and thick-walled and, when examined at a low magnification, are shown to have a number of twisted, enlarged abnormalities. Because of its commercial significance the cotton fiber has been the subject of the most extensive microscopical investigations, and considerable evidence has been gathered as to the mechanism of its growth and the details of both microscopic and submicroscopic structure.

BALLS (7), in 1919, showed that concentric layering becomes visible in the swollen walls of cotton fibers treated with sodium hydroxide and carbon bisulfide. It was found that there is only one thin primary layer while the hair is growing in length but that, as soon as thickening of the wall sets in, the number of layers increases day by day up to a maximum of twenty-five when thickening is complete. BALLS thought that, since growth was arrested by sunshine in the middle of each day in Egypt, the number of these layers corresponded to the number of nights during which the growth in thickness of the cell wall had continued. These concentric layers were thus considered to be rings of nightly growth, differentiated by heterogeneity like the annual rings in timber. These rings are coarser, more sharply demarcated, and less regular in fuzz hairs than in lint hairs, but the later rings in fuzz hairs may resemble those of lint hairs.

BALLS and HANCOCK (8), in 1921, found that every growth layer of the cell wall had a spiral structure and that the pattern of the spiral which appears to be predetermined during growth in length is preserved through all the growth rings of the secondary-wall thickening. They advanced the further theory that the pattern (direction, reversal, and pitch) of these spirals seems to be the major determinant of the externally visible convolutions of the hair.

DENHAM (12), in 1922, followed this work further, indicating that, in striations which occur in all parts of the hair and in all layers of the wall, the direction followed is not always the same in superimposed layers. He corroborated BALLS's idea that the convolutions follow the direction of the primary-wall striations and stated that the cause of the convolutions is a double spiral line of weakness. Excellent photomicrographs were shown of the so-called "slip planes" in the external layer of the fiber.

In 1937 KERR (20) investigated the structure of growth rings in the secondary wall of the cotton hair. In contradiction to BALLS and HANCOCK, who had pictured each growth ring as a separate cylinder or shell of cellulose, KERR concluded that the secondary wall is a complex and coherent matrix and that the appearance of layering in swollen fibers results from a structural differentiation of the cellulose into alternating bands of compact, strongly birefringent zones with more porous, comparatively isotropic lamellae. He found also that during the period of secondary-wall deposition two lamellae, one compact and one more porous, are deposited every 24 hours, and that these two lamellae constitute a daily growth ring. From microscopical observations in which stains were used, it was assumed that compact zones of growth rings are deposited dur-

ing the day and the porous zones at night. Evidence was offered that variations in density are correlated with fluctuation in temperature, the more porous areas being deposited in periods when the temperature dropped below 20° C.

The following year (1938) ANDERSON and KERR (4) showed, in work reported on cotton fibers in various stages of development, that the cell diameter is reached soon after the hair originates but that elongation continues for from 15 to 20 days. Their conclusions as to fiber structure of the seed hair of cotton were summed up as follows:

> The cell wall is composed of
>
> 1. A primary wall containing cellulose and pectic substances.
>
> The cellulose micelles in this wall are grouped into delicate anastomosing threads which have at least two systems of orientations:
>
> (*a*) a flat right-hand spiral, (*b*) a flat left-hand spiral and probably also (*c*) a transverse position. All three systems seem uniform over the entire surface of the fiber cell.
>
> 2. A secondary wall composed of many lamellae of cellulose. The lamellae are not separated from one another by noncellulosic substances but represent dense and less dense areas of cellulose. The layers are formed of systems of spirally wound branching threads and the direction of the spiral is reversed at frequent intervals.
>
> 3. Frequently the pattern of spirals first appearing in the secondary wall is not similar to that in subsequent layers of the wall. Most of the layers of the wall, however, follow a pattern that is established soon after secondary thickening has begun.[2]

In 1941 HOCK, RAMSAY, and HARRIS (18) corroborated much of the previous work of ANDERSON and KERR. They found the cell wall of cotton fiber to consist of a primary and secondary wall. The latter comprises the bulk of the fiber and is made up of innumerable spirally oriented cellulose fibrils inclosed by a winding which also makes a spiral, but in the opposite direction from the former. Both winding and fibrils reverse their direction at frequent intervals along the axis of the fiber, their points of reversal being coincident. The secondary wall is inclosed by a thin primary wall made up of fine crisscrossing strands of cellulose, imbedded in a membrane consisting principally of wax and pectic substance. They stated that, when cotton fibers are swollen under certain conditions, a lamellate structure is discernible in the secondary wall and that the number of these lamellae increases with the age of the fiber.

In 1946 KERR (21) published photomicrographs to show the distinction between the primary-wall membrane and the first ("winding") layer of the secondary wall in undried mature fibers. He stated that in swelling both layers behave so as to restrict the expansion of the cell-wall thickening.

STRUCTURE OF FIBERS OTHER THAN COTTON

Among the bast fibers which have been investigated microscopically for structure, flax has received the most intensive study. In 1922 NODDER (27) published an account which included drawings of the microscopical features of flax, jute, and hemp and photomicrographs of flax, ramie, and hemp. The fibers had previously been swelled with strong solutions of calcium chloride and disintegrated by pressure on the cover slip of the slide. The concentric layering of the walls of these fibers and the spiral arrangement of "fibrils" of which they are composed were clearly shown.

ANDERSON (3), in 1927, studied flax fibers by swelling in 50% sulfuric acid and iodine, and the internal structures were made visible by pressure on the

[2] Quoted with the permission of the copyright owner, the American Chemical Society.

coverslip. His photomicrographs show, first, that ballooning occurs in the early stages of the swelling because of constriction owing to failure of the outer sheath to swell; second, that the various lamellae of the wall may be slipped from one another; and, third, that the outer sheath appears to be made of rather wide spirals. In speaking of the layers of the cell wall, ANDERSON rejected the conception that the dark lines between the layers are made of actual material but stated that these lines merely mark the boundaries between successive lamellae.

In 1942 HOCK (17), in a paper on the microscopic structure of flax as compared with the other bast fibers, presented photomicrographs of cross sections and longitudinal views of the fibers swollen in cuprammonium hydroxide to show the lamellate nature of the cell wall, the spiral nature of fibrillae, and the "ballooning" resulting from constrictions of the outermost layers of fibrillae. He stated that the fibrillae of the outermost layer of the secondary wall spiral in a direction opposite from that of the fibrillae in all the other layers of the secondary wall. He attributed to ramie and flax an identical fibrillate structure, namely, an S twist on all layers except the outermost layer, the fibrils of which have a Z twist. In hemp and jute, on the other hand, he found that, although the fibrils of the outermost layer have the Z twist, the fibrils of the inner layers lie almost parallel to the axis of the fiber. His photomicrographs of flax fibers show concentric rings in swollen cross sections, parallel lamellae in longitudinal view, the actual separation of wall layers by excessive swelling, and the ballooning caused by constrictions of the unswollen outer wall.

Structures similar to those shown in cotton and flax have been demonstrated in various wood pulps by swelling of both lignified and purified fibers. RITTER (31, 32), by alternate treatment with phosphoric acid and alcohol, has demonstrated balloons, cell-wall lamellae, and a banded outer sheath in elm and similar structures in delignified spruce fibers (30). His photographs of this phenomenon are similar to those shown by both NODDER and HOCK for flax. RITTER stated further than the outer layer of the wood fiber is actually composed of tiny threadlike structures which may be unwound. When this layer has been dissolved, an extreme transverse swelling of the inclosed layers of the wall occurs, and balloon-like structures are formed. He pointed out that such a contrast in the behavior of the two kinds of wall layers strongly suggests a difference in structure.

Similar structures were demonstrated by both ALDABA (1) and NODDER (27) for ramie and by BAILEY and KERR (6) for the tropical species *Olmediella betschleriana* and *Pandanus odoratissimus*.

LÜDTKE (23) also showed balloons in lignified pine-wood tracheids which were indistinguishable from similar balloon structures in cotton fibers, and BIXLER (9) presented photomicrographs of spruce-wood fibers taken from paper pulp, which, when swelled with cuprammonium hydroxide, give ballooning.

The concentric rings of cell-wall layers in swollen cross sections of fibers were shown by BAILEY (5) for pine and larch in a delignified fiber swollen in dilute cuprammonium hydroxide and also for a lignified larch fiber swollen with 75% sulfuric acid. Typical ballooning, parallel layers of the cell wall, and a wrinkled lumen wall were demonstrated by HOCK and SEIFRIZ (19) for purified paper fibers (presumably spruce wood) treated with 85% phosphoric acid. NAKASHIMA (25)

has shown similar ballooning for cotton, Manila hemp, and wood-pulp fibers swelled in cuprammonium hydroxide.

CARPENTER and LEWIS (10), working on fibers from paper pulp, followed the changes in the structure of wood fibers as the result of cooking and bleaching operations. Photomicrographs of the typical wall pattern of "outer sheath" and balloons were presented for aspen, spruce, and jack-pine fibers. The only previous work reported on the cell-wall details of milkweed bast is that of DIPPEL (13), which contains no photographs. He included line drawings which are corroborated by the photomicrographs accompanying the present report.

Material and methods

The milkweed fibers were from the pericycle of unretted stems of the giant milkweed (*A. syriaca*) from which they were separated by boiling in water and in 3% sodium hypochlorite. They were examined both in the raw and in the bleached condition.

The cottons examined contained samples from all three of the main categories of typical commercial growths—Asiatic (*Gossypium herbaceum*), Upland (*G. hirsutum*), and Sea Island (*G. barbadense*)—and included both lint and fuzz fibers.

The milkweed and cotton fibers were studied by swelling with such reagents as concentrated sulfuric acid, phosphoric acid, trimethylbenzyl-ammonium hydroxide, or cuprammonium hydroxide. The most satisfactory was found to be cuprammonium hydroxide of the composition specified by the Committee on Viscosity of Cellulose of the American Chemical Society (2) (30 gm. of copper and 165 gm. of ammonia per liter), diluted to about one-third strength. The fibers were stained, then mounted in water, and the swelling agent drawn under the cover slip by means of filter-paper wicks. If swelling proceeded too rapidly, the action was checked by dilute alcohol or alcohol–acetic acid.

No set schedule of histological procedures can be stipulated, since fibers of different degrees of maturity react differently. The strength of the reagents is determined empirically; during swelling they may be diluted by the addition of ammonia to avoid loss of the specimen through solvation. Care and patience in manipulation of the swelling agent to induce separation of the fiber elements without distortion or rupture are required.

Because of the transparency of the swollen fiber, much of the visible structure is difficult to photograph; and staining, therefore, was used to demonstrate the different structures. The stains used for bringing out the primary and lumen walls as distinct from the layers of cell wall which make up the bulk of the fiber were ruthenium red, methylene blue, congo red, or safranin. In the secondary wall layers staining was of no avail. In many fibers the lumen membrane takes the same stains as does the outer sheath; and the protoplasmic residue, when present, is made conspicuous by its affinity for these dyes. However, no specific information as to chemical composition of the different cell-wall layers is gained from staining.

In addition to raw fibers swelled with cuprammonium hydroxide, fibers were also examined from samples which had been pretreated by oxidation with nitrogen dioxide gas or by purification to α-cellulose by boiling with sodium hydroxide. These chemical treatments served to render the cell-wall elements more readily susceptible to swelling reactions, making it possible to see certain

of the structures more clearly than in the untreated fibers.

Discussion

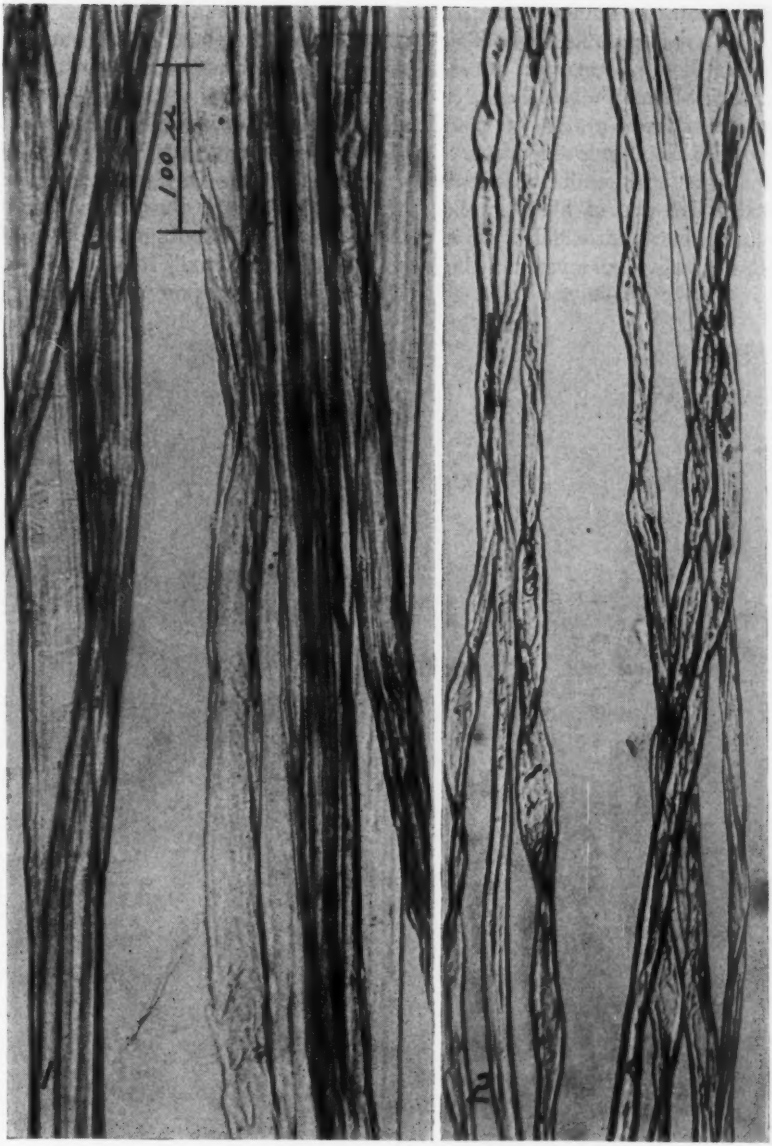
In the individual bast fiber of milkweed the single cell is roughly cigar-shaped with round-pointed ends and, in the desiccated stem, has become collapsed and wrinkled at many points along the fiber length (fig. 1). The fibers range from 15 to 50 mm. in length and from 12 to 30 μ in width, tapering to 1 or 2 μ at the points. The seed hairs of cotton (fig. 2) are much more convoluted and considerably finer in diameter, ranging from 9 to 24 μ in diameter and from 20 to 50 mm. in length, depending on the variety of cotton. There seems to be no outstanding difference in gross structural design between cottons of different varieties or growths or between lint and fuzz fibers.

Ballooning

When swelled in cuprammonium hydroxide, both milkweed and cotton fibers expand rapidly and burst open, the pressure exerted by the cellulose of the swelling wall being great enough to rupture the outer sheath of the fiber. This outermost layer is a thin transparent membrane which, when broken, often rolls up on itself to form tight strings or floats off in large flakes (figs. 3, 4). The rapidly swelling cellulose of the layers of the secondary wall forces itself through the ruptured areas of the primary wall but is prevented from complete swelling by the unbroken portions which form constricting bands. Most frequently the outer sheath breaks at rather regularly spaced intervals, and the consequent swelling of the inner cellulose into "balloons" (fig. 6) accounts for the "beading" or "pearling" figures commonly reported in cotton literature. Similar photographs have been published for flax by Hock (17), for spruce and aspen by Carpenter and Lewis (10), and for Manila hemp by Nakashima (25). In the balloons of milkweed bast (fig. 5) may be seen also the distinct layers of the secondary wall which were inclosed by the outer sheath, and their twisted condition at the constrictions is some evidence of the independent action of each individual layer.

In raw cotton the wall layers are so numerous and so closely packed that they are seldom observed inside the balloons (fig. 6) but may be plainly seen in later stages of the swelling, as in the fuzz fiber of figure 4. Although the number of layers in the secondary wall of the milkweed bast appears to be only five or six, a cotton fiber may have as many as twenty-five (fig. 7), depending on the age in days of growth. Pretreatment of the fibers with gaseous nitrogen dioxide (33), followed by swelling with cuprammonium hydroxide, demonstrated the layering of the secondary wall and a spiraling rupture of the outer sheath (figs. 8, 9). The large number of layers in the cotton fiber and their swelling almost to the point of fusion before they can be discerned as distinct layers are contrasted with the fewer number of readily separable layers in the milkweed fiber. Also seen in these figures are the spiral wrapping in both fibers, into which the outer sheath splits, and the prominent lumen at the center of the cotton fiber brought out by staining with methylene blue.

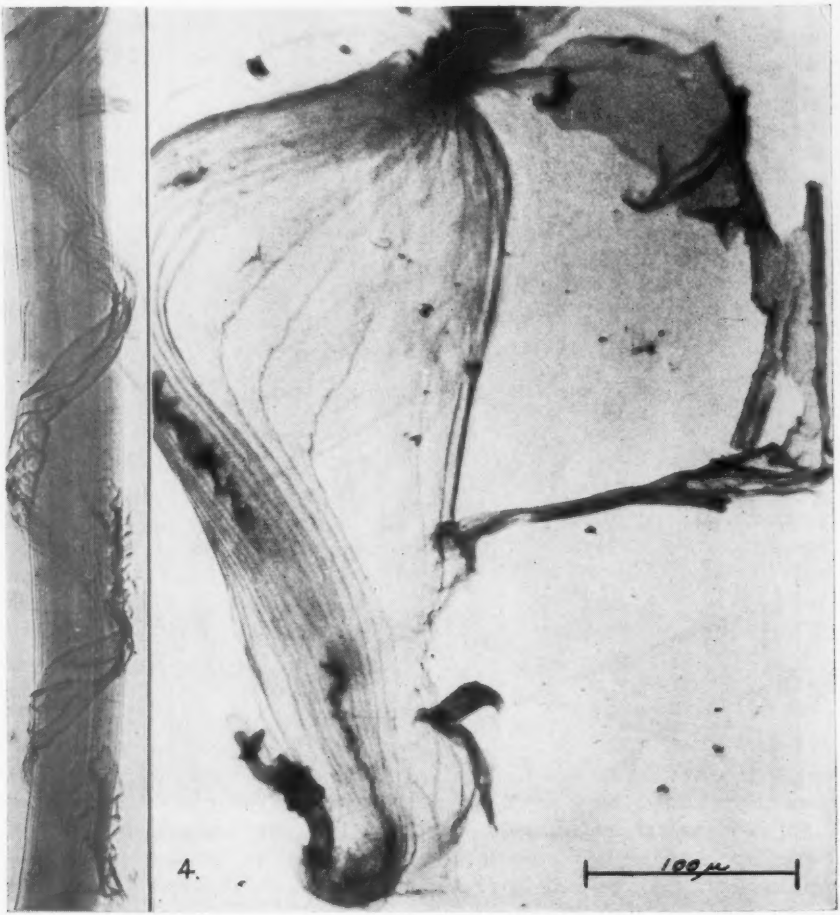
That the outer sheath of the fiber is somewhat elastic is indicated by its rolling-back upon itself when ruptured (figs. 10, 11). A comparative estimate of the extent of swelling of the outer sheath and of the inner cellulose layers shows that "magnification" of the inner walls resulting from swelling in cuprammonium hydroxide solution is in this case approximately eight times. Again the dark-stain-



FIGS. 1–2.—Untreated fibers. Fig. 1, milkweed bast. Fig. 2, cotton lint

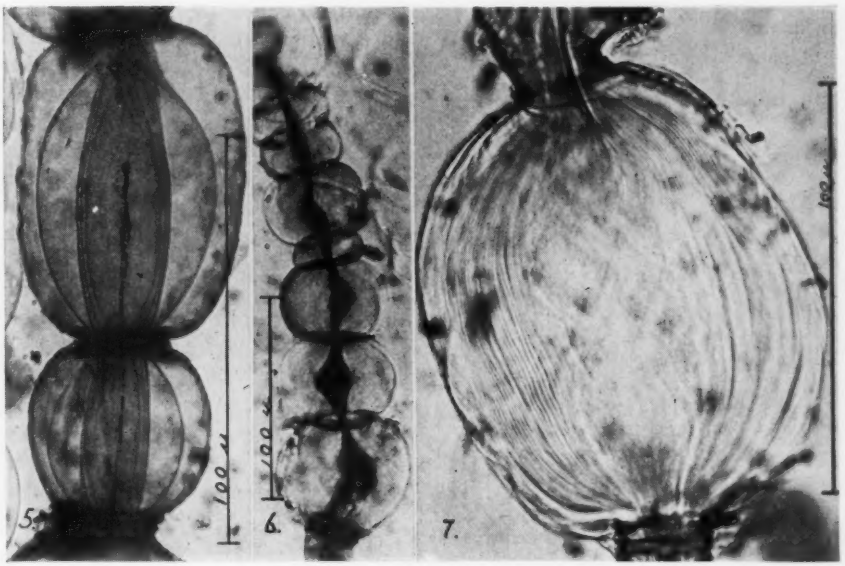
ing residue in the lumen of the cotton fiber (fig. 11) is prominent. It is significant that these photographs are similar to those published previously for cotton (**7, 14, 16**), spruce (**9**), elm (**31**), and an unidentified wood-pulp fiber (**19**).

Separation of the wall layers is plainly shown in the tips of fibers swollen in cuprammonium hydroxide (figs. 12, 13). The tips of individual layers may be seen telescoped one inside the other like a nest of thimbles. In the cotton fiber may be seen a fragment of the outer sheath tip which formerly inclosed all these layers of the secondary wall; and the large lumen with its heavy residue is even more strikingly prominent. Such photographs as these are difficult to explain by KERR's theory of the "alternating bands of compact and porous cellulose forming a coherent matrix." It is, of course, possible that the porous cellulose which



FIGS. 3–4.—Fibers swollen to show rupture of outer sheath. Fig. 3, milkweed bast. Fig. 4, cotton fuzz

joined these layers (20) has been dissolved by the swelling agent. On the other hand, the photographs appear to support the theory of ALDABA (1), who, in his work on flax and ramie, found the cell wall to be formed by a series of telescoping tubular membranes which gradually become transformed into cell-wall lamellae. Figures 14 and 15 show portions of milkweed and cotton fuzz fibers, respectively, where the wall layers seem to be discrete.



FIGS. 5–7.—Ballooning of swollen fibers caused by constrictions of unbroken outer sheath. Fig. 5, milkweed bast; note twisted and wrinkled condition of inner layers of secondary wall at constrictions. Figs. 6, 7, cotton lint.

LUMEN WALL

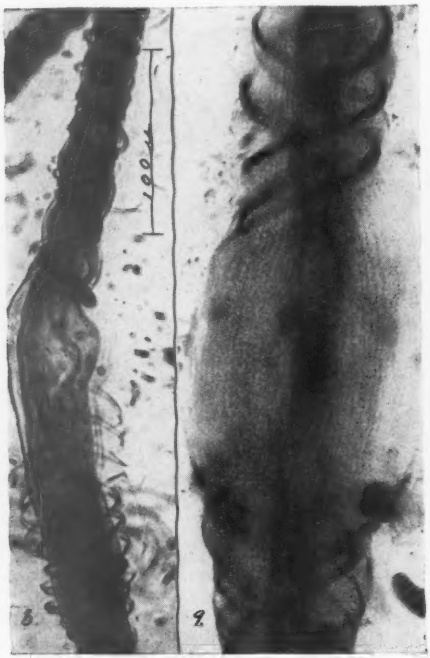
The layer lining the lumen often behaves differently from the other wall layers. It resists swelling and frequently stains similarly to the outer sheath of the fiber. The fibers shown in figures 16 and 17 had been pretreated with nitrogen dioxide gas before swelling with cuprammonium hydroxide. The oxidized secondary wall has fused so that no lamellate structure is discernible, but the more resistant lumen wall has not yet swelled enough to unwrinkle the shriveled membrane of which it is composed.

Figure 18 shows swollen cross sections of a group of milkweed bast fibers, remarkably similar to the cross section of an onion. Although fibers of many different ages are represented, some of which were cut near the tip and others at the widest portion of the fiber, it is obvious that the maximum number of layers in any fiber is seven. Figure 19 shows a cotton fiber cross section similarly swelled to show the numerous fine wall layers in the secondary wall. Further photomicrographic evidence of these characteristic lamellae was presented by KERR (20) for cotton, by BAILEY (6) for

larch and pine, and by HOCK (17) for flax.

OUTER SHEATH

Figures 20 and 21 show a single milkweed bast fiber swollen in cuprammonium hydroxide and photographed at two different levels of focus. In figure 20 may be seen the inner wall layers inclosed within the outer sheath or primary wall. Figure 21 shows the top surface of the outer sheath and indicates that it has a definite structure of spiral bands. It is not clear whether the banding is the actual pattern of the outermost sheath or whether it is a pattern induced in the continuous outer membrane by a spiral structure beneath it, which, though separate, swells enough to cause its rupture in a spiral manner. As one follows the course of the spiral, it would appear that it winds in a double ribbon to cover the surface of the fiber.



FIGS. 8–9.—Chemically treated fibers swollen to show spiral winding of outer sheath inclosing lamellae of secondary wall. Fig. 8, milkweed bast. Fig. 9, cotton lint.

Figures 22, 23, and 24 show spiral bandings in the outer sheath of cotton lint fibers which resemble the structure for milkweed bast (figs. 20, 21). Evidence that these bands are not chemically induced artifacts is given by photomicrographs of similar structures in pine fibers by CARPENTER and LEWIS (10), in cotton by DENHAM (12), LAWRIE (22), CLEGG (11), and OSBORNE (28), in flax by NODDER (27), and in spruce by RITTER (32).

Recently MITCHELL (24), in a paper on the nitrated cellulosic constituents of wood, has published photomicrographs of cell-wall "lignin residues" produced in the swelling of nitrated wood fibers with ethyl acetate which show the same spiraling pattern of double bands.

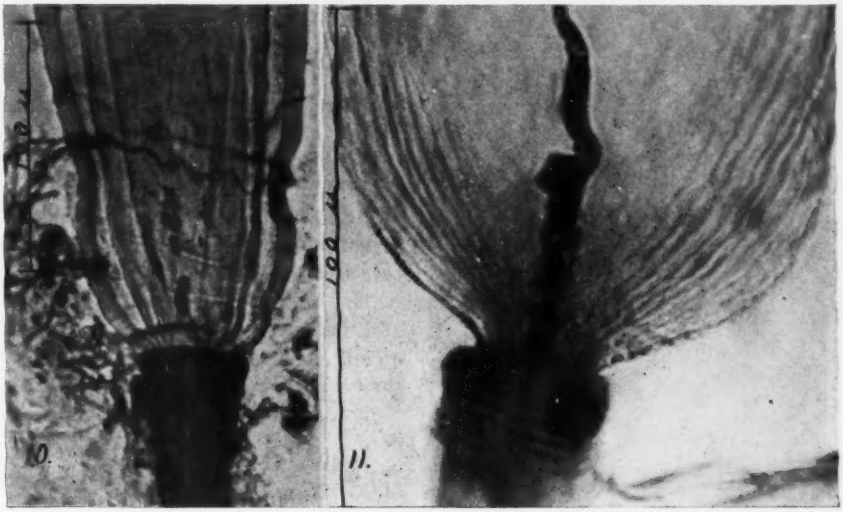
It has not been determined whether the spiral banding apparent in figures 22–24 is a structure of the outer sheath of the fiber or is a pattern in the primary wall caused by the swelling of a spirally wrapped thread just beneath it. It would appear from figure 23, however, that the spiral band is a pattern of the outer sheath itself. This unusual view of a cotton fiber was obtained by oxidation of the fiber in nitrogen dioxide gas for the excessively long treatment of 74 hours, after which the major part of the fiber was readily dissolved in dilute cuprammonium hydroxide, while an outer portion remained more or less intact as a solid shell. Evidently the prolonged treatment so deteriorated the cellulose of the secondary wall that it exerted no swelling pressure to rupture the fiber sheath. On the other hand, in the un-

treated fuzz fiber (fig. 4), swelling pressure of the layers of the secondary wall was so great as to rupture the primary sheath longitudinally rather than in spirals.

In milkweed the spiral banding is more plainly shown in figure 25, in which three of the inner layers of the fiber have escaped through a rupture in the outer sheath to leave the spiraled tip an empty shell.

The chemically treated cotton fiber in figure 26 shows the stained primary sheath pared away to disclose the transparent swollen cellulose of the secondary wall in parallel lamellae on either side of the lumen.

Although in dried cotton it has not been possible to photograph the spiral direction of the finer structural elements of which each lamella is composed (cf. ANDERSON and KERR [4]), figure 27



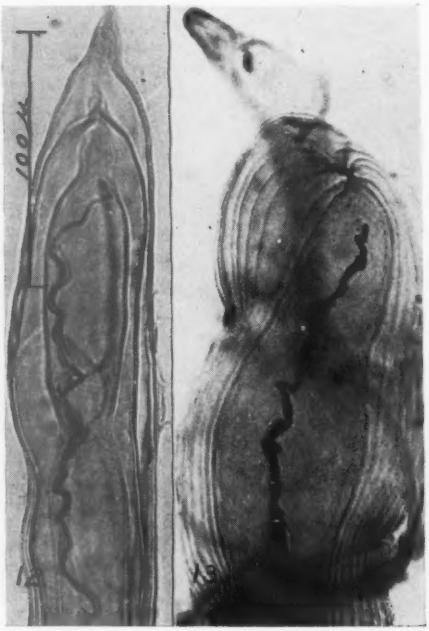
FIGS. 10–11.—Fibers swollen to show lamellae issuing from inclosing primary sheath. Fig. 10, milkweed bast. Fig. 11, cotton fuzz fiber. Note rolled sheath at base of swollen portion.

Although it has not been possible to photograph for cotton, as was done for milkweed (figs. 21, 25), the lamellae inclosed within the spiraling outer sheath, it is evident from figures 22–24 that a similar structural design exists for both types of fibers. In figure 24 the unswelled portion of the cotton fiber protected by the primary sheath is stained with methylene blue, and the broken sheath can be seen separating into a spiral wrapping.

shows the fine spiral threads in a wall layer of a milkweed bast fiber lying at an angle to the fiber axis. KERR (21) has recently photographed this same phenomenon in mature but undried cotton fibers from a green boll. It must be assumed that refractive indices in dried cotton fibers are so changed that photographic evidence is extremely difficult to obtain or that swelling obscures this fine structure by fusing the fibrils into a solid mass.

Kapok and milkweed floss, though

technically seed hairs and morphologically parallel to cotton, did not respond to swelling solutions in the typical manner described in this paper. This was undoubtedly because they lack sufficient layers of cellulose to produce swelling pressures great enough to rupture the outer wall. The presence of waterproofing materials deposited in the outer sheath



FIGS. 12–13.—Closed tapered tips of swollen fibers. Fig. 12, milkweed bast. Fig. 13, cotton fuzz fiber. Note cell-wall layers emerging from ruptured primary sheath.

was also a contributing factor, though milkweed floss fibers from which the wax had been extracted failed to swell in cuprammonium hydroxide.

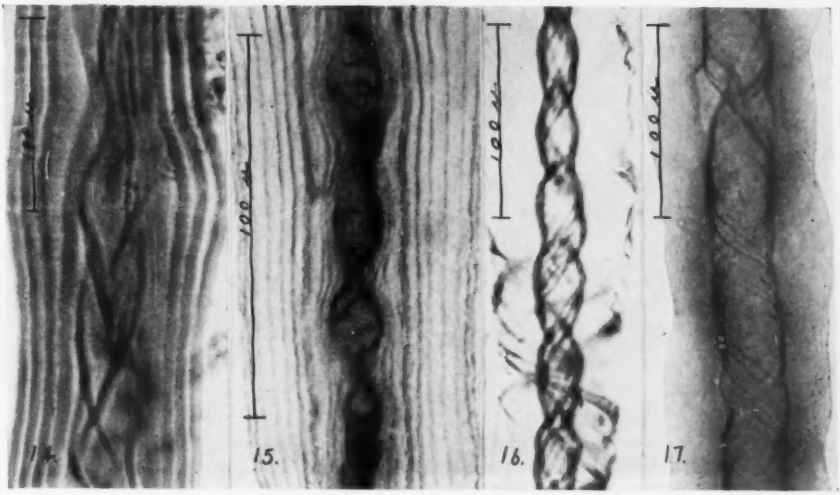
Summary

1. The similarity between two widely different types of fibers such as milkweed bast and seed hair of cotton illustrates a basic principle of nature in the structural design of cell walls of the fibrous type.

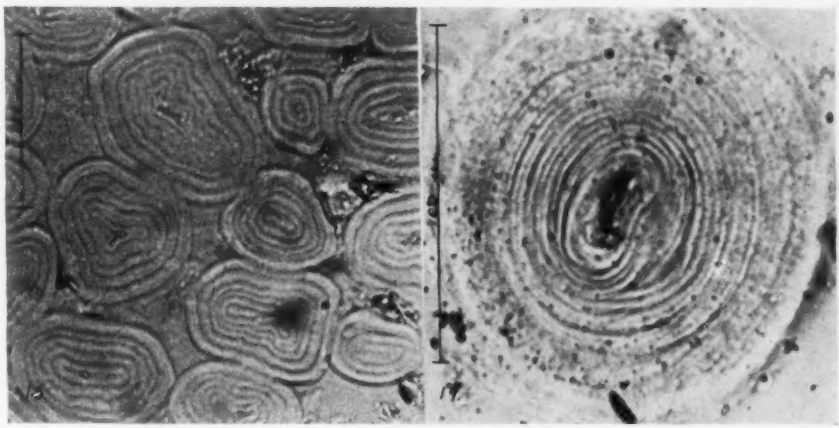
2. The photomicrographs here presented support in general the findings of other workers in the field of microscopical cell-wall structure. It is established that the cell wall consists of (*a*) an outer sheath, which is hardly capable of any appreciable swelling; (*b*) a many-layered cellulose wall which may be swelled ten to twenty times its original size before solvation; and (*c*) an inner canal inclosed by a lumen wall which in its reactions is more like the outer membrane of the fiber than it is like the secondary-wall layers. The fiber sheath appears to have a banded structure when not ruptured by swelling of the cellulose beneath it. In addition to confirming previous observations, these photomicrographs indicate that the cell-wall layers of the secondary wall in both seed hairs of cotton and milkweed bast fibers are discrete membranes, readily separable one from the other by swelling reagents. The photomicrographs further serve to co-ordinate information acquired by various investigators working on individual fibers from diverse natural sources.

3. Although no spiral structure was observed in any layer of the secondary wall of cotton, it is probable that the spiraling fibrils are so fine as to be fused by the swelling action or are rendered indistinguishable by their refractive index. Spiral fibrils were photographed in a layer of the secondary cell wall in a nitrogen dioxide–treated milkweed fiber after swelling in cuprammonium hydroxide.

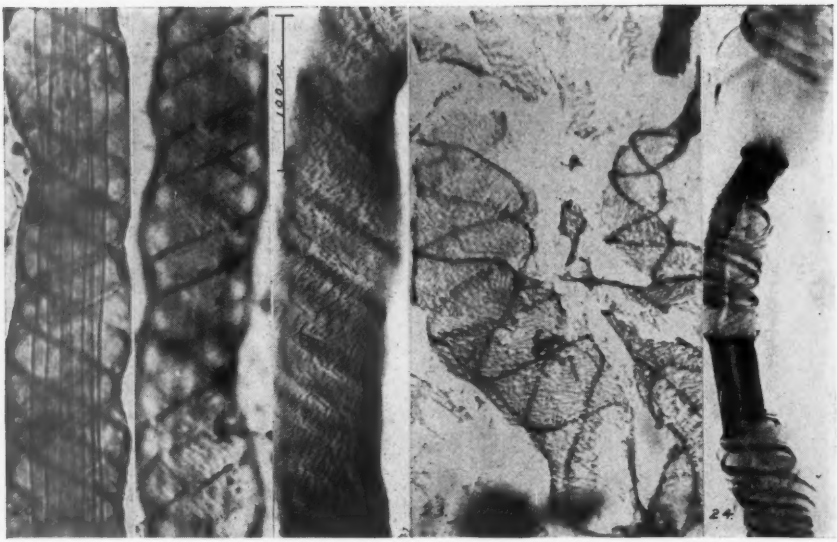
The author wishes to express her appreciation for the interest and advice of Mr. T. L. W. BAILEY, JR., in the details of the microscopical work; and for the



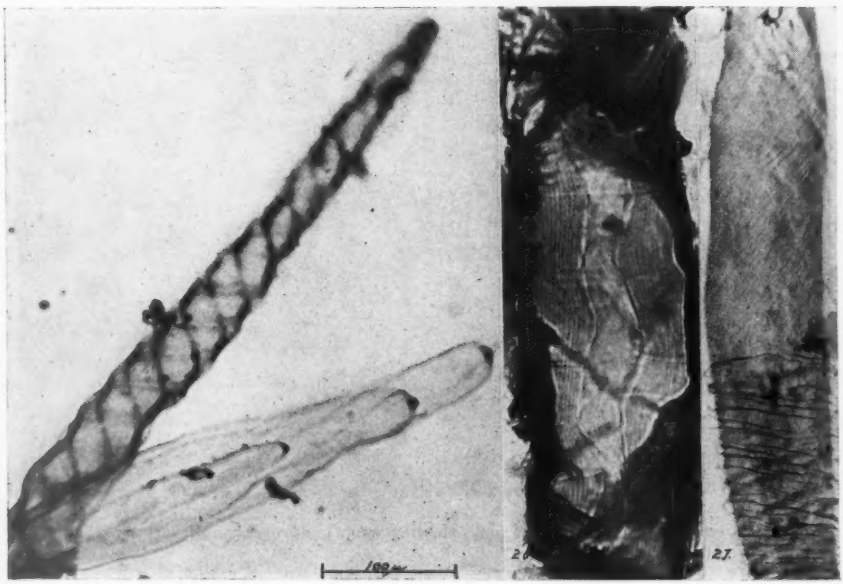
FIGS. 14–17.—Fibers swollen to show layers of secondary wall and convoluted lumen. Fig. 14, chemically treated milkweed bast. Fig. 15, cotton fuzz fiber. Fig. 16, chemically treated milkweed bast. Fig. 17, chemically treated cotton lint.



FIGS. 18–19.—Swollen cross sections of fibers showing layers of secondary wall. Fig. 18, milkweed bast fibers. Fig. 19, cotton lint.



FIGS. 20–24.—Fibers swollen to show banding in outer sheath. Figs. 20, 21, milkweed bast with focus at center and top planes, respectively. Figs. 22, 23, 24, chemically treated cotton lint. Fig. 22, focus at top plane. Fig. 23, residue after solvation of fiber. Fig. 24, uneven rupturing of outer sheath into spirals.



FIGS. 25–27.—Chemically treated fibers swollen to show inner structure. Fig. 25, milkweed bast. Note inner layers released from ruptured spiral sheath. Fig. 26, cotton lint. Note lamellae disclosed by peeling back of ruptured sheath. Fig. 27, milkweed bast. Note fibrillar structure of cell-wall layer beneath broken spiral of outer sheath.

assistance of Miss INES V. DE GRUY in photomicrography and in the preparation of the manuscript. Acknowledgment is also due Dr. R. E. REEVES and his co-workers for the nitrogen dioxide treatment of the fibers.

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PERSISTENCE OF 2,4-DICHLOROPHENOXYACETIC ACID IN SOIL IN RELATION TO CONTENT OF WATER, ORGANIC MATTER, AND LIME[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 583

OLIVE H. KRIES

Introduction

The increasing use of 2,4-dichlorophenoxyacetic acid (2,4-D) in spray applications to plants for various purposes and the possibilities in expanding its use to include direct treatment of soil necessitate the investigation of its possible effects upon and persistence in soils of diverse types in relation to various management practices. Experiments reported upon to date (**1, 4, 5, 6, 7, 8, 11, 12**) indicate that the length of persistence in soil of the toxic effects to higher plants varies with soil type and management as well as with the amount applied, the method of application, and the kind of plant.

The present experiment was devised to secure additional data on the persistence of activity of 2,4-D in one soil in relation to its content of water, organic matter, and lime. It was carried on in the University of Chicago greenhouses from March 27 to August 31, 1946.

Material and methods

Heavy black loam from northern Illinois was mixed with coarse sand in the proportion of 3:1 by volume. Approximately 1440 pounds of the mixture were divided into six lots, two of which were allowed to become air-dry; the other four lots were kept moist. Prior to drying, partially decomposed leaf mold was added to one of the lots to be air-dried and to two of the moist lots in the ratio of 1:3 by volume. Lime ($CaCO_3$), at the rate of 1 lb. to 500 lb. of soil, was mixed with two of the four moist lots on March 27. The six lots thus included: dry soil; dry soil + leaf mold; moist soil; moist soil + leaf mold; moist soil + lime; and moist soil + lime + leaf mold. These terms will be used in referring to these lots hereafter.

After preparation all six lots were stored in large boxes in a room adjacent to the greenhouse. The reactions of the various combinations ranged from pH 7.8 to 8.2; moisture equivalents, determined by the centrifuge method, from 19.2% to 21.5%. Water content in the moist lots ranged from 21.5% to 24.3% during the first week of storage.

Meanwhile, 36.0-, 3.6-, and 0.36-gm. quantities of commercial grade 2,4-D were each dissolved in 100 ml. of ethyl alcohol. The three solutions were each then thoroughly mixed by hand with

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

1-kg. amounts of white quartz sand which was spread out to allow evaporation of the alcohol.

The six different lots of stored soil were each then divided into four aliquots. To three aliquots of each lot, the dry sand containing 2,4-D was added on April 7 (11 days after the lime had been added) so as uniformly to incorporate 2,4-D into the soil at the rate of 2, 20, and 200 p.p.m., respectively, based on the dry weight of the soil. The fourth aliquot of each lot served as an untreated control. The amount of sand so added was negligible in its effect upon textural and structural relations of the soil.

The twenty-four lots of soil which resulted from the possible combinations of the various treatments were placed in storage in a room not subject to great temperature fluctuations; temperatures 2-4 inches below the surface in the stored soil averaged about 74° F. with a low of 58° on April 26 and a high of 84° on July 8.

Water was added to the moist lots occasionally to offset evaporational losses but not in such quantity as to result in leaching.

On April 14 and 21, May 5, July 17, and August 15, soil from each lot was placed in 5-inch, white, glazed pots provided with glass-wool filters over the drainage hole. Torpedo sand filled the lower one-fourth of each pot. On April 14 and 21 ten pots were so filled from each of the twenty-four stored lots, and on the other dates five pots were filled from each. Thus, soil was taken from each storage lot into the greenhouse 1 week, 2 weeks, 4 weeks, 14½ weeks, and approximately 18 weeks after 2,4-D had been mixed with it.

On each removal date the filled pots were placed on a greenhouse bench, and each pot was uniformly planted with five Red Kidney beans and ten seeds each of barley, African marigold, and white mustard. These species are sensitive in different degree to 2,4-D. The rate and percentage of emergence of the seedlings were used as primary criteria of the degree of toxicity of 2,4-D and of its persistence in the different stored lots on the various planting dates.

Half the number of pots in each treatment in the April 14 planting were replanted on May 12. In this process the soil was allowed to dry to the point where the old plants could be pulled free with little loss of soil. The other pots in the April 14 planting were replanted on June 2. A third planting was made on July 15 on the soil initially planted on April 14 and replanted on May 12.

Similarly, half the number of pots of the April 21 sowing were replanted on May 19. In the cases of two pots of each of the twenty-four treatments, the top and bottom halves of the soil mass were removed to clean, separate pots to test whether residual toxicity differed in the top and bottom layers of the soil.

On August 16 the pots initially planted on July 17 were replanted.

Ten days after each planting all but one of the bean seedlings in the control pots were cut off below the cotyledons so that the smaller seedlings would not be excessively shaded. This procedure was not necessary in the first plantings in the pots containing 2,4-D because of its inhibiting effect on beans.

Counts of germination were made 14 days after each planting. Germination was considered, following PORTER (9), as the emergence and development from the embryo of those essential structures which, for the kind of plant in question, were indicative of the ability to produce a normal plant under favorable conditions. All such data were calculated in percentages and graphed (figs. 1–4). Further development of the plants was fol-

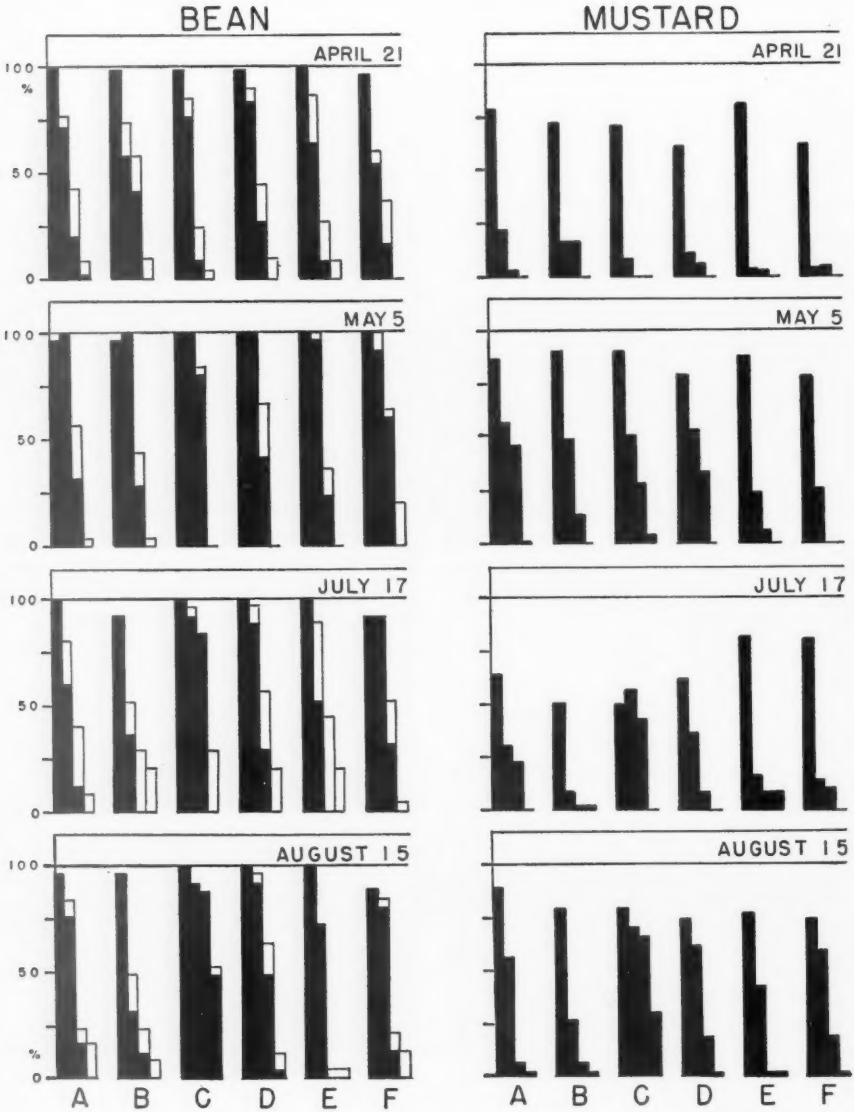


FIG. 1.—Percentages of emergence of test plants 14 days after planting on indicated dates from soil which had been stored since April 7. Conditions of storage were: *A*, dry soil; *B*, dry soil + leaf mold; *C*, moist soil; *D*, moist soil + leaf mold; *E*, moist soil + lime; *F*, moist soil + lime + leaf mold. Each group of four bars, from left to right, respectively, represents emergence from soil in which 2,4-D had been incorporated on April 7 at rate of 0, 2, 20, and 200 p.p.m.

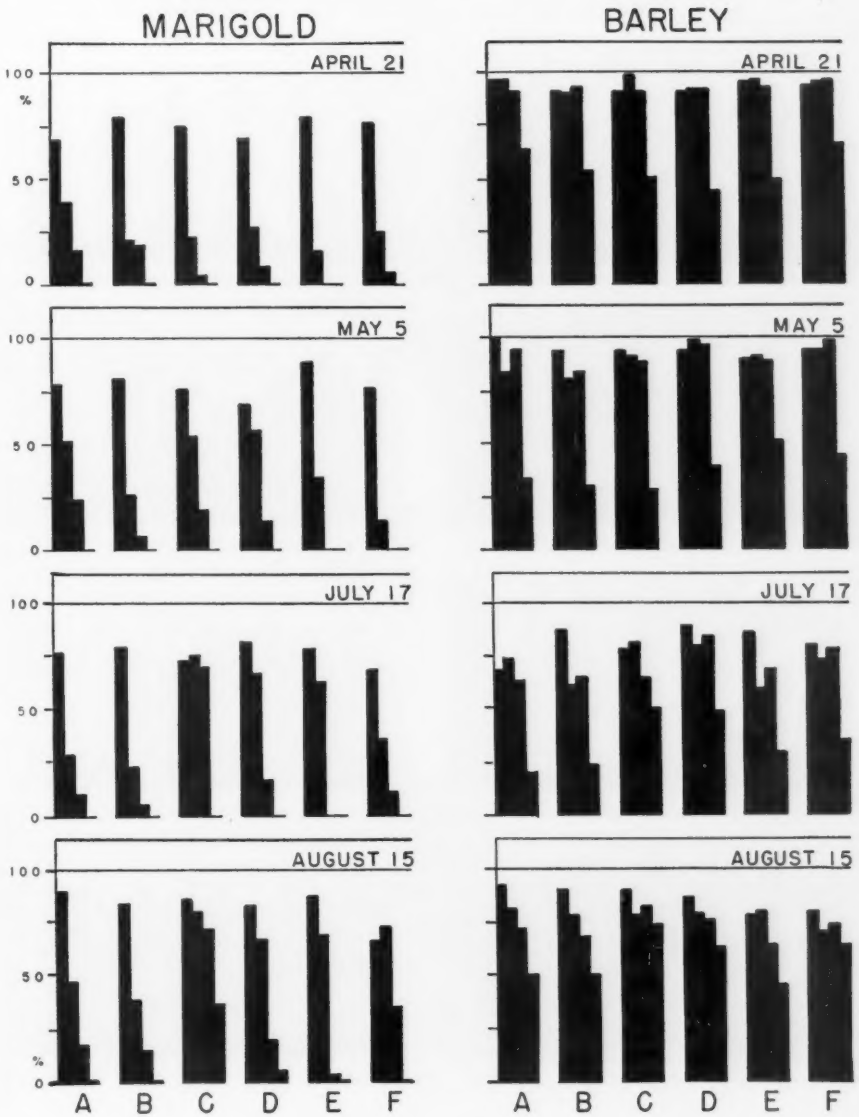


FIG. 2.—As in fig. 1 for other test species

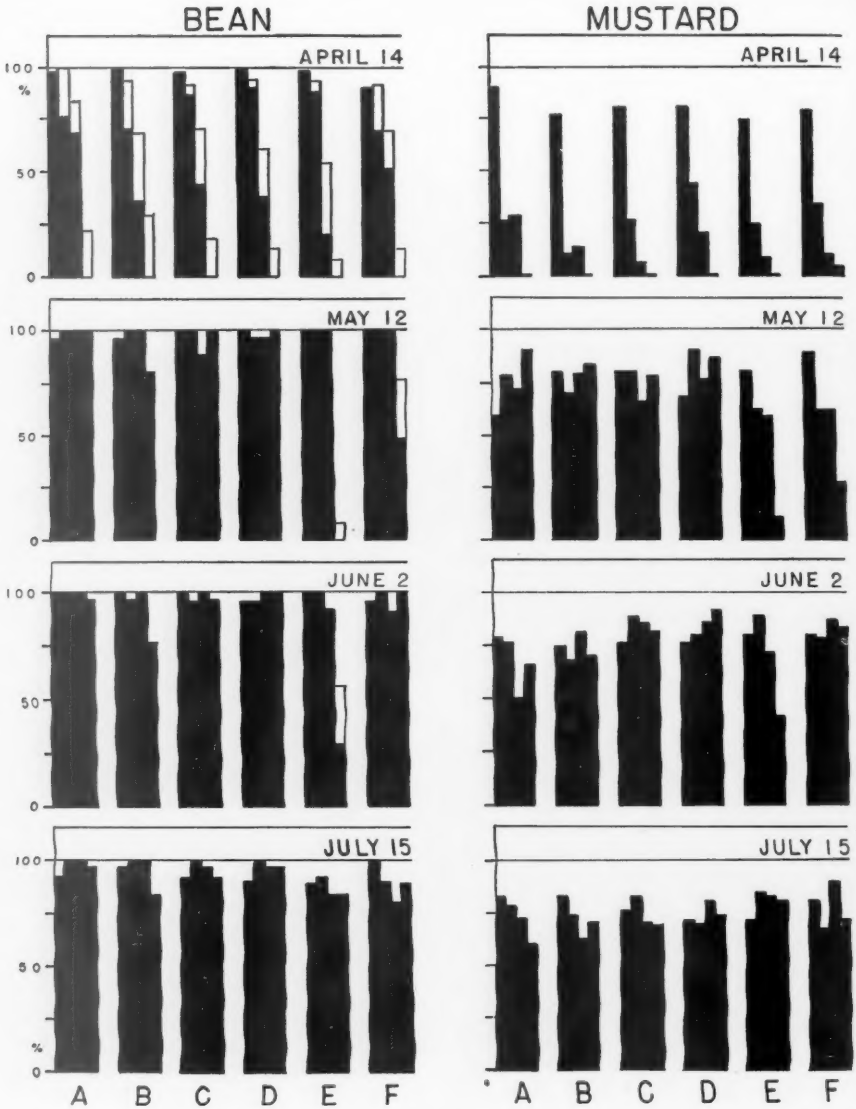


FIG. 3.—Percentages of emergence of test plants 14 days after planting on indicated dates. Each group of four bars and categories *A*, *B*, *C*, *D*, *E*, and *F* as in fig. 1. Planting on April 14 made after 1 week of soil storage. Data for other dates are of replantings on same soil following 4, 7, and 13½ weeks of cropping. Note effect of lime (*E*) on retention of toxicity and of leaf mold (*F*) in mitigating its effect.

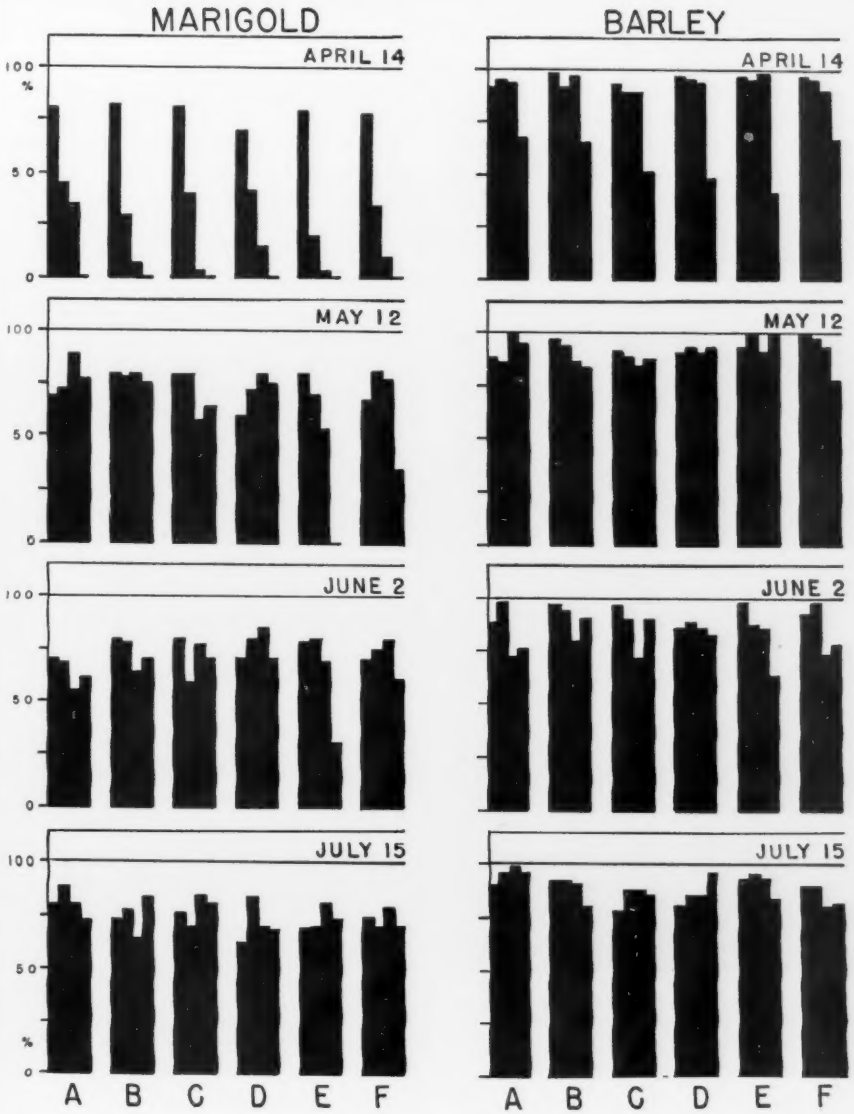


FIG. 4.—As in fig. 3 for other test species

lowed for varying lengths of time after each planting date.

The pots were watered sparingly but frequently so as to avoid leaching of 2,4-D in drainage water. Thus, the eventual loss of toxicity either from the stored soil or from the soil after its removal to the greenhouse and planting was due in part, at least, to factors other than leaching.

FIG. 5.—Beans 26 days after planting on May 5 in moist soil + lime. *Left*, from 2-p.p.m. (2,4-D) pot; *right*, from 20-p.p.m. pot.

Air temperature in the greenhouse from April 21 until the last week in May varied from 90° to 98° F. at midday to a night minimum of about 74°. During the last week in May and practically all of June, daily maximum readings stood at 98° with one exception. During the first part of the third week of June, temperatures remained close to 80° day and night. A low of 65° occurred twice during the month. Through July 15, noon temperatures reached 100° on an average with a low on one night of 76°. In August, daily maximum temperatures were somewhat lower, with an average of about 95° and one low of 74°.

Naturally, the plants required large amounts of water to offset losses by transpiration during these continued high temperatures. Frequent but light watering was maintained. Soil temperatures in the pots at a depth of 4 inches ranged from 59° to 98° F. during the season.

Results

SOME EFFECTS OF 2,4-D ON SEEDLING DEVELOPMENT

As judged by seedling emergence in this experiment, mustard and marigold seeds were more intolerant of 2,4-D than Red Kidney bean and barley, and marigold seemed to be more sensitive than mustard. The sensitivity of marigold to 2,4-D has been previously reported (2, 3).

Development of bean seedlings deviated from normal to such an extent in all the initial plantings on soils originally containing 20 and 200 p.p.m. of 2,4-D that it was necessary to designate the seriously injured specimens in making the counts. Consequently, the clear spaces in the bar graphs (figs. 1, 3) indicate the percentages of seedlings showing markedly abnormal development, the solid bars those with more nearly normal development. The seriously injured seedlings were often upside down and much contorted (fig. 5). Frequently, they possessed four flanges of tissue at the bases of the hypocotyls. These abnormalities have been described by others (1, 4).

The capacity of substituted phenoxyacetic acids to induce modifications in size, shape, pattern, and venation of leaves was first reported in 1942 (13). In the present experiment the heart-shaped leaves of beans strongly affected by 2,4-D often failed to expand properly and were yellowish-green in color with the veins more or less outlined with small black dots. In general, they appeared stiff and dry. Some of the trifoliate leaves in any later plantings although the irregularities appeared in some of the 2,4-D treatments in all the successive initial plantings on stored soil.

Mustard germinated first in each planting, and those mustard seedlings which emerged from soil treated with 2,4-D exhibited no characteristic abnormalities.

Marigold seedlings developed swollen regions at the bases of the petioles in

FIG. 6.—First "trifoliate" leaves of beans from various 20-p.p.m. (2,4-D) pots in April 14 planting

of bean displayed telemorphic effects (2, 3) from 2,4-D in the production of excessive numbers of leaflets as well as in the nondivergence of leaflets. Some leaves had one leaflet only, while others had as many as six or eight divisions (figs. 5, 6). No specimens were found with more than ten leaflets per leaf. Judging from counts made on these anomalous leaves of bean, more plants in the first sowing (April 14) in the soil treated with 2 and 20 p.p.m. of 2,4-D possessed this type of malformation than some plants in the initial plantings in soil originally containing 20 p.p.m. of 2,4-D. Occasionally, the leaves seemed narrower than those in the controls. If germination was possible, however, there were no striking morphogenic effects from 2,4-D on either marigold or mustard.

In barley the coleoptiles remained very pale, almost white, for several days in the initial plantings in soil originally containing 200 p.p.m. of 2,4-D. Later, the green plumules emerged weakly and elongated

a few inches. A brown spotting superficially resembling a rust developed on the leaves of plants in the 2- and 20-p.p.m. soils in most of the initial sowings in stored soil. Frequently it was followed by a chlorosis of the entire leaf. A burning of the leaf tip was also rather common in these pots.

Germination in control pots

Some deviations in the percentages of germination in the controls were apparent in relation to season of planting, but the six major soil categories had little effect on the percentages of emergence in control pots on any one planting date. Throughout the experiment Red Kidney bean was high in percentage of germination in all controls. The percentage of germination for mustard was noticeably reduced in the July 17 planting, while the percentage for barley was relatively low in both the July 17 and the August 15 plantings. On the whole, of the four species tested, marigold was lowest in percentages of germination in controls for all ten planting dates. All statements concerning the effects of 2,4-D upon emergence are based on comparisons with the control pots in the same category of soil.

Inhibitory effects of 2,4-D in stored soil

The persistence of the toxic effect of 2,4-D in stored soil is shown in table 1, in figures 1 and 2, and in the data for April 14 in figures 3 and 4, based chiefly on percentages of emergence of test species in the five different plantings on stored soil. The data in table 1 are weighted somewhat in accord with the later development of the seedlings.

Results from soil stored 1 week (figs. 3–4 for April 14) and from soil stored 2 weeks (figs. 1–2 for April 21) were practically identical. Toxicity to bean, marigold, and mustard was apparent at all three levels of concentration of 2,4-D in all six categories of soil treatment. For barley there were no inhibitory effects in the soil originally containing 2 and 20 p.p.m. of 2,4-D in any of the six combinations.

Results from the planting on May 5 (figs. 1–2), after 4 weeks of soil storage, were much the same as in the previous plantings, except that Red Kidney bean showed little or no injury in the 2-p.p.m. soil, and barley appeared to be stimulated in the 20-p.p.m. dry-soil treatment.

In the July 17 planting on soil stored for 14½ weeks, there were no pronounced inhibitory effects on any of the four species in the moist-soil lots at the 2- and 20-p.p.m. levels. All the seeds in the moist-soil lot at 200 p.p.m. were affected, however. The other five major soil categories showed toxicity essentially the same as in the three earlier initial plantings on stored soil.

In the last of the initial sowings on August 15, on soil stored 130 days, pronounced inhibitory effects continued with some exceptions: the lots of moist soil were least toxic of all, and moist soil+leaf mold, with or without added lime, was less toxic than the other three soil combinations.

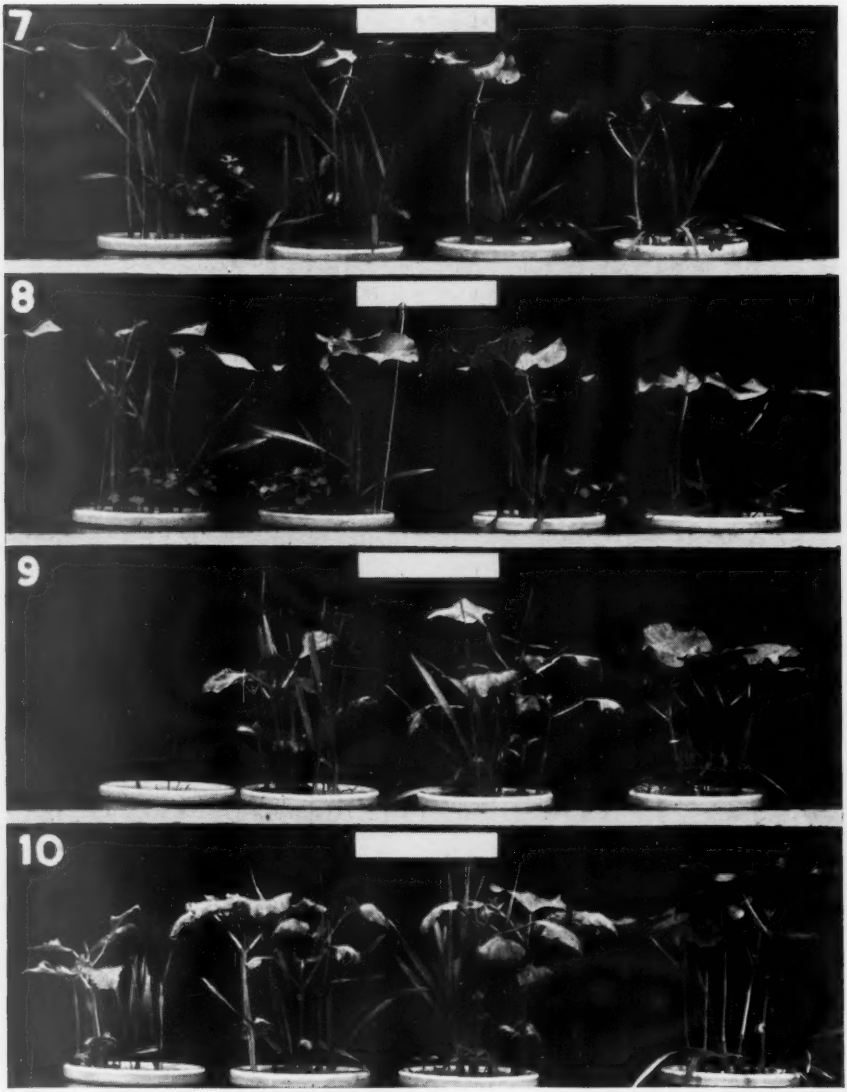
Thus, in general, there was very slight difference among soil lots stored for 1, 2, or 4 weeks in the persistence and degree of toxicity of equivalent amounts of 2,4-D added at the beginning of storage. The persistence was little affected by moisture content of soil, by the status of its lime content, or by the addition of leaf mold. After 14½ weeks of storage, however, there had been a pronounced loss of toxicity in the moist-soil lots which contrasted strongly with the five other categories. There was a gradual

TABLE 1

PRESENCE OR ABSENCE OF TOXIC OR STIMULATING EFFECTS WHICH RESULTED FROM MIXING 2,4-D IN SOIL ON APRIL 7 AS SHOWN BY SEEDLING EMERGENCE AND LATER DEVELOPMENT OF FOUR TEST SPECIES*

INITIAL SOIL	2,4-D ADDED (P.P.M.)	SOIL IN STORAGE APRIL 7–14; THEN IN GREENHOUSE				SOIL IN STORAGE APRIL 7–21; THEN IN GREENHOUSE		SOIL IN STORAGE APRIL 7–MAY 5; THEN IN GREENHOUSE	SOIL IN STORAGE APRIL 7–JULY 17; THEN IN GREENHOUSE		SOIL IN STORAGE APRIL 7–AUGUST 15; THEN IN GREENHOUSE
		Planted April 14	Replanted May 12	Replanted June 2	Replanted July 15	Planted April 21	Replanted May 19	Planted May 5	Planted July 17	Replanted August 16	Planted August 15
Dry soil	2	+++−	−−−−	−−− S	S−− S	+++−	−−−−	−++−	+++−	−−−−	−++−
	20	+++−	− S S S	−−−−	S−− S	+++−	− S−−	+++ S	+++−	+−−−	+++−
	200	++++	− S− S	−−−−	−−− S	++++	+−−−	++++	++++	++++	++++
Dry soil+leaf mold	2	+++−	−−−−	−−−−	S−−−	+++−	−−−−	−++−	+++−	−−−−	+++−
	20	+++−	−−−−	−−−−	S−−−	+++−	−−−−	+++−	+++−	−+−−	+++−
	200	++++	−−−−	−−−−	−−−−	++++	+++−	++++	++++	+++−	++++
Moist soil	2	+++−	−−−−	−−−−	S−−−	+++−	−−−−	−++−	−−−−	−−−−	−−−−
	20	+++−	−−−−	−−−−	−−−−	+++−	−−−−	+++−	−−−−	−−−−	−−−−
	200	++++	−−−−	−−−−	−−−−	++++	−−−−	++++	++++	++±−	+++−
Moist soil+leaf mold	2	+++−	− S S−	−− S−	S− S−	+++−	− −S−	−++−	−++−	−−−−	−−+−
	20	+++−	− S S−	−− S−	S− S−	+++−	− S S S	+++−	+++−	−−−−	+++−
	200	++++	− S S−	−−−−	S− S S	++++	− S−−	++++	++++	−+−−	+++−
Moist soil+lime	2	+++−	−++−	−−−−	−−−−	+++−	−++−	−++−	++++	−−−−	+++−
	20	+++−	−++−	−−−−	− S S−	+++−	+++−	+++−	++++	+++−	+++−
	200	++++	+++−	+++−	− S−−	++++	++++	++++	++++	+++−	++++
Moist soil+lime+leaf mold	2	+++−	−+−−	−−−−	−−−−	+++−	−+−−	−++−	−++−	−+−−	−−−−
	20	+++−	−+−−	−−−−	− S S−	+++−	−+−−	+++−	+++−	−+−−	+++−
	200	++++	+++−	−−+−	−−−−	++++	++++	++++	++++	+++−	+++−

* From left to right, each group of entries indicates effect on bean, mustard, marigold, and barley, respectively: "+" indicates obvious toxicity; "S" shows obvious stimulation; "−" indicates no effect. Data are based on comparison with plants in control soil similarly treated except lacking 2,4-D.



FIGS. 7–10.—Test plants on May 25 in replanting of May 12. *Left to right*, pots with initial concentrations of 200, 20, 2, and 0 p.p.m. of 2,4-D mixed in soil. Fig. 7, moist soil; fig. 8, moist soil + leaf mold; fig. 9, moist soil + lime; fig. 10, moist soil + lime + leaf mold.

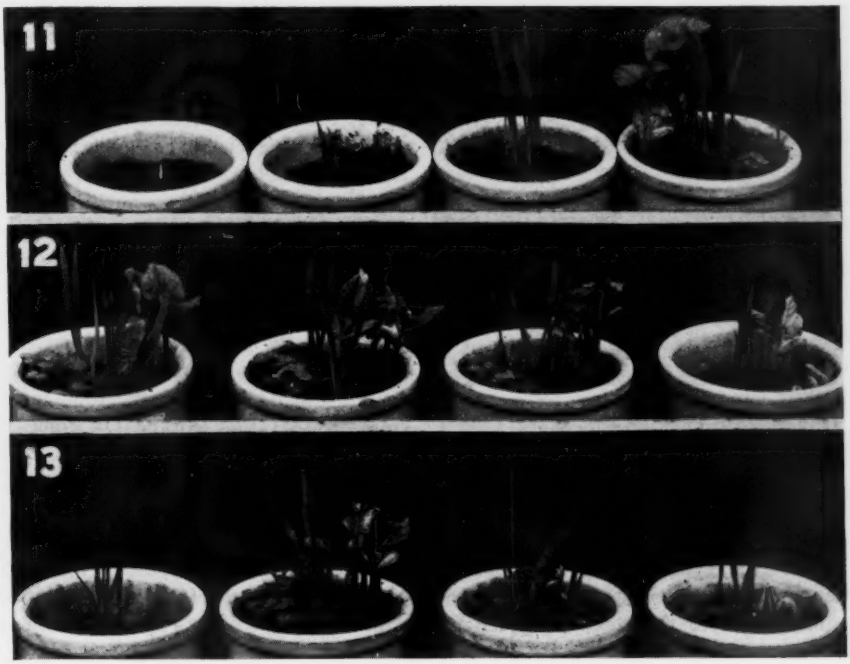
diminution of toxicity in the lots of moist soil+lime+leaf mold as shown by the plantings of May 5, July 17, and August 15 in contrast with strong persistence of toxicity in the moist soil+lime combination.

Inhibitory effects in replanted soil

As shown by the replanting on May 12 in soil which had been cropped in the greenhouse for 4 weeks, toxic effects from 2,4-D had completely disappeared except in the limed lots of soil (figs. 7–10). In the latter, some toxicity to one or more of the test species was still present at all test levels of 2,4-D (table 1, figs. 3, 4), although generally in lower degree than in the April 14 planting. The presence of partially decomposed leaf mold, however, had markedly reduced the toxic condition in the limed soil, especially in the case of marigold, and for bean in the 200-p.p.m. soil (figs. 9, 10). Some apparent stimulation of growth was evident in mustard, barley, and bean in the lots of dry soil with initial concentrations of 20 and 200 p.p.m. of 2,4-D, and also in mustard and marigold in all levels in moist soil+leaf mold (fig. 8).

A replanting on June 2 of soil which had been cropped in the greenhouse for 7 weeks showed strong inhibition in the 200-p.p.m. moist soil+lime, except in the case of barley, although the toxicity was somewhat less than on May 12. As in the May 12 replanting, the presence of leaf mold had further reduced the toxic condition in the limed soil.

In the July 15 replanting in soil which



Figs. 11–13.—Test plants on May 25 in replanting of May 19. *Left to right*, pots with initial concentrations of 200, 20, 2, and 0 p.p.m. of 2,4-D mixed in soil. Fig. 11, moist soil + lime; fig. 12, top half of soil mass in planting of April 21, moist soil + leaf mold; fig. 13, bottom half of soil mass.

had been cropped in the greenhouse for $13\frac{1}{2}$ weeks, toxic effects from 2,4-D had completely disappeared in all treatments, including the limed soils. Moreover, there was some apparent stimulation to growth of all test species, in comparison with controls, in some of the lots which had been treated with 2,4-D (table 1).

In comparing the replantings of May 19 and August 16 with the replanting of May 12 (table 1), all three of which were made on soil which had been cropped in the greenhouse for 4 weeks, it appeared

FIG. 14.—Test plants on June 10 in replanting of May 19 in soil originally planted on April 21, moist soil + lime + leaf mold. *Left*, bottom half of soil mass in April 21 planting; *right*, top half.

that the May 19 replanting practically repeated the results of May 12, except in the case of dry soil+leaf mold, which remained toxic to the three dicotyledons in the 200-p.p.m. soil. In the resowing of August 16, deleterious effects of 2,4-D had apparently been more persistent than in the other comparable replantings, since there was still pronounced toxicity to one or more test species in all soil originally containing 200 p.p.m. of 2,4-D. Possibly the very high greenhouse temperatures during the August 16 test intensified the activity of plants in response to the presence of the growth-regulator, or for various reasons it had not been lost from the soil or inactivated as rapidly.

In the replanting on May 19 of the upper and lower portions of soil from pots which had been cropped in the greenhouse for 4 weeks, a strong contrast in residual toxicity between top and bottom portions, as shown by seedling emergence and later development, occurred only in the 200-p.p.m. lots (figs. 11–14). The lower half was definitely more toxic to mustard and marigold, but Red Kidney bean in this portion also showed symptoms of toxicity in misshapen leaves and yellowish-green color in the seedlings. The effect of leaf mold in diminishing the toxicity of 2,4-D in the limed soil was well shown in this planting (figs. 11, 14).

These data suggest that some of the relatively greater decrease in toxicity of the top layers may have resulted from the downward leaching of 2,4-D from the soil in the pots, in spite of the care used in watering, especially since it disappeared much less rapidly from moist stored soil. HANKS (6) and DE ROSE (4) have both shown that 2,4-D may be leached rapidly from some soils.

In conclusion, in soil lots lacking added lime, 4 weeks of cropping in the greenhouse generally sufficed to eliminate the toxicity of all the different original levels of 2,4-D. There was, however, strong residual toxicity in the limed lots in all three replantings which followed 4 weeks of cropping. Some toxicity in the 200-p.p.m. treatment persisted in the limed soil after 7 weeks of cropping, and possibly more, but disappeared completely between the replanting dates of June 2 and July 15. In each replanting test the presence of partially decomposed leaf mold had offset, to a considerable extent, the effects of the added lime in delaying the loss of

toxicity, especially in the 2- and 20-p.p.m. levels.

Discussion

The striking persistence, after cropping for 4 weeks, of the toxic effects of 2,4-D on mustard and marigold in the limed soil originally treated with 2, 20, and 200 p.p.m. of 2,4-D, and on Red Kidney bean at the 200-p.p.m. level, is regarded by the writer as the most significant result of the experiment. In an experiment on leaching 2,4-D from soil, HANKS (6) found that its inhibitory effects persisted longer in a naturally alkaline, sandy clay soil, pH 8.4, than in any other of the six soils tested. He found, however, that addition of lime to these six soils seemed to produce no significant differences in toxicity of comparable leachates as tested by a slight modification of the corn-root bio-assay developed by SWANSON (10). In confirming the results of the bio-assay, HANKS tested Red Kidney bean emergence and development and observed that the alkaline soil mentioned was the only one showing toxic effects of 2,4-D to bean at the end of 6 weeks of leaching. HANKS's experiment was based on an original 20-p.p.m. level of 2,4-D mixed in the soil in contrast to the concentrations of 2; 20, and 200 p.p.m. used in the writer's experiment.

TAYLOR (11), working with two soils, pH 7.8–7.9 and pH 6.9–7.0, found that dicotyledonous weeds were almost completely eliminated for the growing season on these medium-light field soils treated at the start of the season with 1–4 lb. of 2,4-D per acre. In general, he found that the development of bean and mustard was more strongly inhibited than that of wheat and corn in proportion to intensity of 2,4-D treatment. He further noted the possible influence of marl ($CaCO_3$) in the soil which was low in organic matter. He reported a persistence as long as 7 weeks of the toxic effects of 2,4-D applied at the rate of 4 lb. per acre. In the greenhouse the writer found that effects of 2,4-D at an initial concentration of 200 p.p.m. continued for 50 days, at least, in the case of the moist soil+lime.

HAMNER *et al.* (5), working with muck soil, reported that the toxic effects of 2,4-D mixed in it did not persist longer than 4 weeks. HANKS (6) found that the leachate from peat so treated was relatively free of toxicity, as measured by corn-root growth, 2 weeks after the incorporation of 2,4-D. In the writer's experiment the presence of large amounts of leaf mold in the soil reduced the effect of added lime in retarding the loss in toxicity.

After 18½ weeks treated soil in storage had retained most of its toxicity at all levels of 2,4-D with one exception, that of moist soil with original concentrations of 2 and 20 p.p.m. of 2,4-D. There was practically no loss of toxicity in soils stored air-dry. MITCHELL and MARTH (7) found a retention of toxicity for as long as 18 months in soil in relatively dry storage.

The more vigorous growth of some seedlings in 2,4-D–treated soil, in contrast to controls, in the replantings of May 12, June 2, and July 15 is noteworthy in many of the soil combinations. This apparent stimulation was greatest in the mixture of moist soil+leaf mold. HANKS found a stimulation of corn-root growth, in contrast with controls, in one or more of the weekly leachates from five of six soils treated with 2,4-D, the exception being the naturally alkaline soil mentioned previously. TAYLOR (11) also reported a stimulating effect on some plantings on soil previously treated with 2,4-D.

It has been demonstrated (1, 4) that germinating seeds are very susceptible to small amounts of certain growth-regulating substances, certain broadleaf species being more sensitive than some cereals. The behavior of Red Kidney bean, African marigold, and mustard in this experiment paralleled these findings for the dicotyledons, while barley showed resistance to 2,4-D in all initial plantings at 2 and 20 p.p.m. The single exception to this statement concerning barley appeared in the planting of July 17, especially on moist soil+lime, in which higher temperatures may have been an important factor in accentuating the toxic symptoms.

Modification of leaf form in bean repeated itself with surprising regularity in initial plantings in the 20-p.p.m. pots and resembled somewhat the morphogenic effects on various leaves described by ZIMMERMAN and HITCHCOCK (13) and by DEROSE (4). The appearance of Red Kidney bean seedlings in the initial plantings on soil containing 2, 20, and 200 p.p.m. of 2,4-D in this experiment closely duplicated those illustrated by ALLARD *et al.* (1).

Leaching, decomposition through action of micro-organisms in the soil, and inactivation due to adsorption or fixation by soil colloids have been suggested as factors contributing to the reduction or loss in activity of 2,4-D in soil (4, 6). The writer's experiment suggests that the status of its lime content and the organic content of soil may strongly affect the rate of loss of toxicity from 2,4-D mixed in the soil, whatever the mechanisms involved. Toxicity was also gradually diminished under some conditions of moist storage of soil in contrast with its retention in dry storage, although conditions in moist cropped soil led to a much more rapid loss of toxicity than did those in comparable moist stored soil. It is thought that reduction of toxicity in stored soil could not have resulted from leaching and that leaching was not the primary factor in the loss of toxicity in cropped soil.

Summary

1. Partially decomposed leaf mold, water, and lime were mixed with heavy black-loam soil from northern Illinois in such fashion as to yield six lots: air-dry soil; air-dry soil+leaf mold; moist soil; moist soil+leaf mold; moist soil+lime; and moist soil+lime+leaf mold. To four equal aliquots of each of the six lots, 2,4-dichlorophenoxyacetic acid (2,4-D) was mixed on April 7 at the rate of 0, 2, 20, and 200 p.p.m., respectively. All twenty-four lots were placed in storage.

2. Portions of each lot were placed in pots on greenhouse benches on April 14, April 21, May 5, July 17, and August 15. Each pot was planted with Red Kidney bean, mustard, African marigold, and barley to test by the emergence and later development of these plants the degree and persistence of toxicity from 2,4- D in the different lots of stored soils.

3. The soil originally planted on April 14 was replanted on May 12, June 2, and July 15 to test for persistence or loss of toxicity in the various treatments under cropping. Soils initially planted on April 21 and July 17 were replanted on May 19 and August 16, respectively.

4. The capacity to induce toxic symptoms was strongly persistent in soil in dry storage as measured by all initial test plantings. There was some diminution of toxicity in some lots in moist storage, although others, especially the limed ones, retained strong toxicity for 18 weeks.

5. In general, as shown by three different replantings, 4 weeks of cropping in

the greenhouse sufficed to eliminate toxicity to all test species from all soil except that which had been limed or to which 2,4-D had been added at the rate of 200 p.p.m. Seven weeks of cropping resulted in the loss of toxicity to all test species from all except the limed soil at the 200-p.p.m. level. Such long persistence of 2,4-D in this treatment suggests caution in its use in calcareous or newly limed soils. In all replantings the presence of large amounts of leaf mold strikingly reduced the degree and persistence of toxicity to one or more test species in the limed soil. Thirteen weeks of cropping resulted in complete loss of toxicity from all pots.

6. In some replantings there was more vigorous growth of seedlings in soil treated with 2,4-D than in the controls. Such stimulation was found in one or more test species in one or more replantings for twelve of the eighteen lots treated with 2,4-D.

7. Sensitivity to 2,4-D in the soil increased in the order barley, bean, mustard, and African marigold. In the initial plantings on soil containing 2 and 20 p.p.m. of 2,4-D, the first trifoliate leaves of bean characteristically showed abnormalities involving nondivergence of the leaflets or their multiplication to numbers as high as ten.

The writer wishes to express gratitude to Dr. CHARLES E. OLMSTED, under whose immediate supervision all phases of the experiment were carried on.

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SOME RESULTS OF CROSS-POLLINATION ON *LILIUM REGALE*[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 584

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One of the most popular of our garden lilies is *Lilium regale* Wilson, commonly known as the regal lily. Because of the many desirable characters which it possesses, numerous attempts have been made at hybridizing it with other species, but they have generally been unsuccessful when *L. regale* was used as the seed parent. Although the application to the stigmas of *L. regale* of pollen from almost any species of *Lilium* may result in the production of a greater or fewer number of viable seeds, the progeny derived through such pollinations, almost without exception, have shown no evidences of paternal inheritance.

Partly in an effort to learn why this result was obtained, a large number of cross-pollinations have been made during the last decade on *L. regale*, both in our local garden and at Wychwood, Lake Geneva, Wisconsin. These experiments were begun in 1936 and have been continued each season, using pollen from *L. amabile*, *L. browni*, *L. canadense*, *L. candidum*, *L. concolor*, *L. martagon*, *L. pumilum*, *L. speciosum*, *L. superbum*, *L. umbellatum*, and *L. willmottiae* (*L. davidi* var. *willmottiae*) as well as from a few hybrids including *L.* × Backhouse, *L.* × Maxwill, *L.* × *elegans*, and *L.* × *testaceum*. In some cases as few as four or five seeds per capsule have developed after pollination, while in others, notably when the pollen of *L. candidum* was used, practically all the ovules developed into mature viable seeds. There was also marked seasonal variation in the quantity of seeds set. For example, the summers of 1936 and 1938 were unfavorable, and comparatively few seeds developed, whereas during 1937 and 1946 heavy sets of seeds developed in at least some capsules from almost all the pollinations.

Seeds resulting from the cross-pollinations were planted in flats in the greenhouses in late November or early December of each year. Usually a high percentage of germination was obtained. The seedlings were allowed to remain in the flats until late May or early June following, when they were transplanted to the garden. For a few years the small bulbs resulting from this first season's growth were sent to the Willamette Valley of Oregon for further development because of the more suitable climatic conditions prevailing there. Owing to transportation difficulties which developed during the war, however, the practice was discontinued after 1941, and the seedlings have since been grown in our local garden.

Both here and in Oregon some of the seedlings flowered in the third season and most of them in the fourth. Regardless of the pollen which had been used on the seed parent, all first-generation progeny appeared to be pure *L. regale*, with no greater variability in flower color or size, leaf form, and size or height of stems than occurred in the progeny of selfed *L. regale*.

A number of flowers from a part of nearly all the first-generation crosses were self-pollinated. Generally the capsules set an abundance of seeds, approxi-

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

mating that of selfed *L. regale* closely in quantity. The seeds showed high viability, and their seedlings were as vigorous as those of *L. regale* seedlings. The time required for flowering in the supposed F_2 plants was the same as for the seedlings of the first-generation crosses or for seedlings from selfed *L. regale*. These supposed F_2 plants still appeared to be pure *L. regale*, showing no segregation and no indication whatever of hybridity.

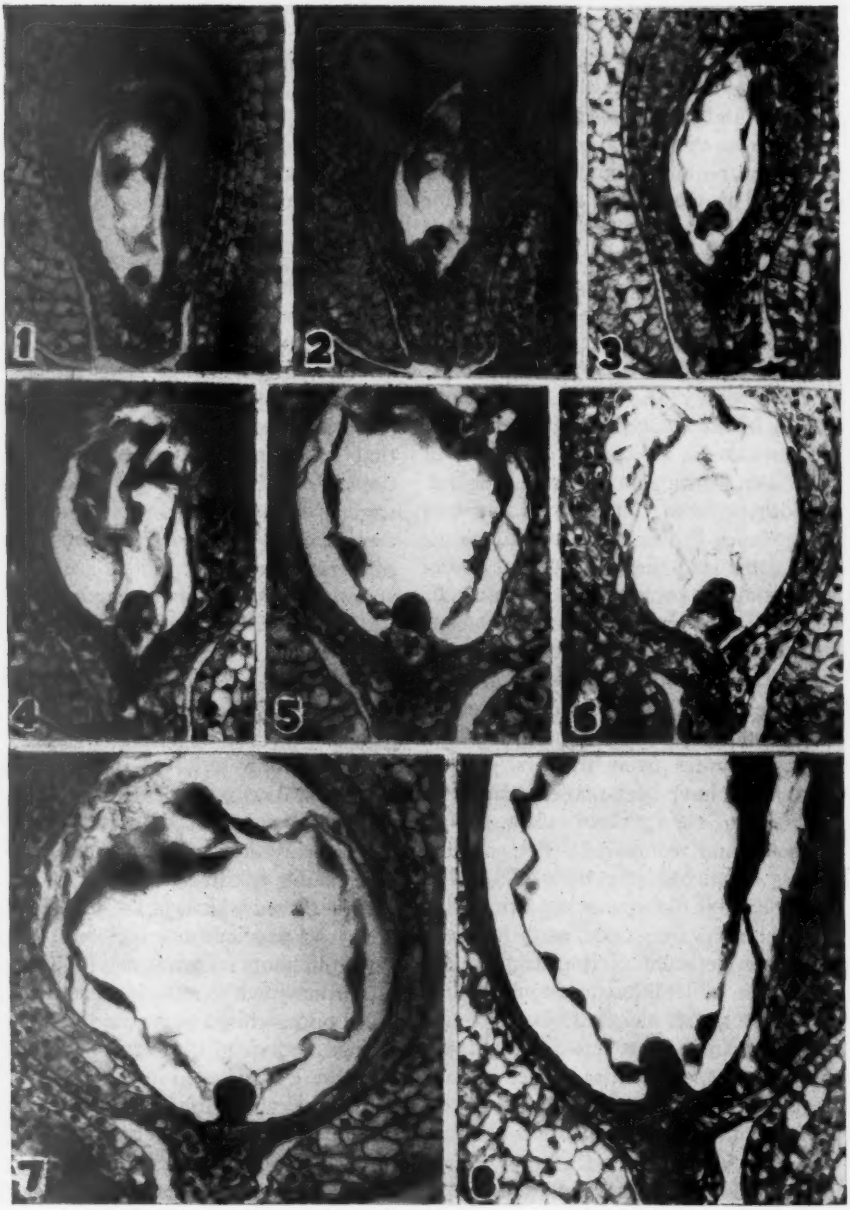
For several years, too, Professor RONALD BAMFORD and certain of his graduate students at the University of Maryland have been working on the same problem. In both personal conversation and correspondence Professor BAMFORD has informed me that their results and observations are in complete agreement with mine. The photomicrographs, which constitute a part of the present paper, were sent to him in order that he might compare them with their preparations.

Several hundred smear preparations of microsporocytes from the first-generation crosses have been made, stained according to the gentian violet–iodine method, and compared with similar smears of microsporocytes of *L. regale*. No observable differences in pairing relations, chiasma frequency, or disjunction have been detected. Neither lagging nor other detectable differences could be observed. In short, meiosis in the progeny resulting from the cross-pollinations showed no peculiarities which would serve to distinguish them from the progeny of selfed *L. regale*.

Ovaries were collected at appropriate intervals during several of the years from both self- and cross-pollinated plants, cut transversely into segments usually 5–8 mm. long, fixed in NAVASHIN's solution, handled according to the tertiary butyl-alcohol method, and sectioned, according to age, at thickness ranging from 20 to 35 μ. Megasporogenesis and megagametophyte development in *L. regale* follow the *Fritillaria* type, according to the results of COOPER (1), and my observations are in complete agreement with his report.

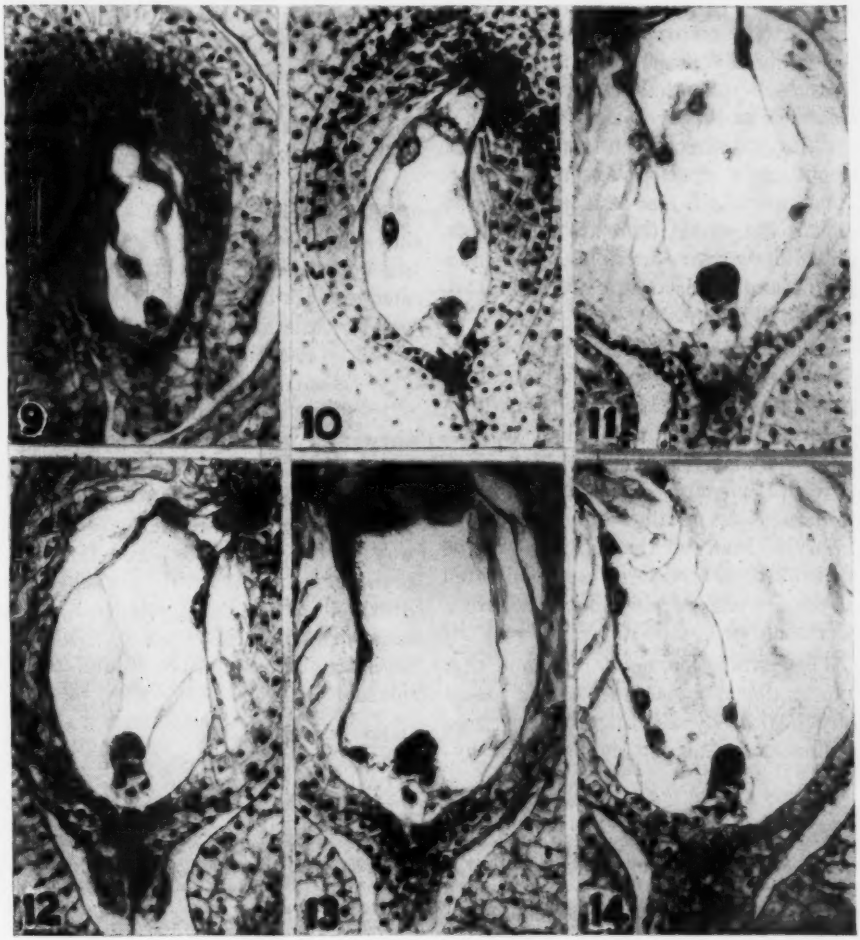
In self-pollinated plants the pollen tubes reached the ovules at the distal end of the ovary in approximately 100 hours, while to reach those at the proximal end sometimes required as long as 120 hours. The time may vary, however, as a result of variable environmental conditions so that, when especially favorable conditions prevail, the ovules nearest the stigmas may show the pollen tubes in their micropyles as early as 96 hours or, conversely, when conditions are not favorable, it may require 120 hours or more. Rather large numbers of ovules were seen which showed the pollen tubes shortly after they had passed through the micropyles with the two microgametes already discharged into the megagametophyte. The microgametes, when first discharged, are elongated and vermiform, as has been noted frequently by other investigators. Various stages in the double-fertilization process have been observed which, in the main, differ little from previous descriptions (3, 4, 5).

At 188 hours following self-pollination the primary endosperm nucleus had divided once, while the zygote nucleus remained undivided (fig. 1). At 216 hours there were four nuclei in the endosperm and a two-celled embryo (fig. 2). At the side of this embryo was a second structure which possibly might be a twin embryo derived from one of the synergids, as has been reported for *Lilium* by COOPER (2). A similar condition has been observed in only a few other sections of the self-pollinated ovaries and in none of those from the cross-pollinated



FIGS. 1–8.—Stages in development of embryo and endosperm in self-pollinated *Lilium regale:* fig. 1, 188 hours, with undivided zygote nucleus and two-nucleate endosperm; fig. 2, 216 hours with apparently twin embryos and at least an eight-nucleate endosperm; fig. 3, 268 hours, with embryo and endosperm only little more developed; fig. 4, 312 hours, with embryo sac starting to enlarge; fig. 5, 360 hours, showing increased volume of endosperm in enlarged embryo sac; fig. 6, 408 hours, with only slight advances over preceding; fig. 7, 456 hours, similar to preceding; fig. 8, 485 hours, endosperm greater in volume and number of nuclei, embryo relatively little farther advanced.

ones. At 268 hours the endosperm appeared to possess at least sixteen nuclei and the embryo several (fig. 5). The volume of the embryo sac next increased noticeably as the volume and number of cells of the endosperm increased (figs. 4–6), but with proportionately less rapid increase in volume of the embryo. The growth of the embryo does not keep pace with that of the endosperm (fig. 7, 456 hours; fig. 8, 485 hours). During the period of approximately 500–900 hours following fertilization, the embryo remains relatively small and little differentiated, according to some other material examined but not reported on herewith, while the endosperm continues to develop rapidly.



FIGS. 9–14.—Stages in development of embryo and endosperm in *L. regale* pollinated with pollen of *L. candidum:* fig. 9, 288 hours, similar to fig. 2; fig. 10, 360 hours, similar to fig. 3; fig. 11, 432 hours; fig. 12, 456 hours, similar to fig. 6; fig. 13, 468 hours, similar to fig. 7; fig. 14, 492 hours, similar to fig. 8.

Although the earliest stages from cross-pollinated plants have been found at 288 hours after pollination, the evidences of pollen-tube entrance through the micropyle were still clear (fig. 9). The endosperm development is comparable with that in selfed plants (fig. 3), but embryo development is possibly a little slower. At 360 hours (fig. 10) development of both embryo and endosperm is comparable with that in selfed plants (cf. fig. 5). The embryo sac next increases markedly in volume (figs. 11–14), just as in the self-pollinated plants, and the appearance of embryos and endosperm is strikingly similar in the two series, with about equal development at the same time intervals. So far as it has been possible to determine, there are no recognizable differences in development of endosperm and embryo in self- and cross-pollinated plants.

Additional studies have been carried out on the cross-pollinated plants, with collections having been made at 1- or 2-day intervals for more than 50 days after pollinations. The ovules, which were nearly mature seeds at that time, contained essentially fully developed embryos. Many of the capsules were as well filled as those on self-pollinated plants. No recognizable differences whatever could be observed.

These studies have so far given neither information nor real clues to the reason why the progeny resulting from the cross-pollinations show no evidences of hybridity. It is planned to continue the experiments during the summer of 1947 and to investigate materials at some of the time intervals following pollinations which are not covered in the present report.

Summary

1. During the last decade cross-pollinations have been made on *Lilium regale* with pollen from more than a dozen species of lilies. Viable seeds have developed in some capsules following all the cross-pollinations. The seedlings resulting from these seeds reach flowering size during the third and fourth seasons following planting, but among several thousand of the supposed F_1 hybrids no evidences whatever of the pollen parent have been manifested; they closely resemble the seed parent in all visible characters. These seedlings show no greater variability than that exhibited by seedlings resulting from self-pollination.

2. Smear preparations of microsporocytes from the supposed F_1 hybrids showed no differences in meiotic behavior when compared with microsporocytes of *L. regale*. Following cross-pollination, the pollen tubes entered the ovules by way of the micropyles, and both embryos and endosperm developed at approximately the same rate and in the same manner as in selfed *L. regale*. No explanation has so far been found for this unusual behavior.

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DEVELOPMENTAL SEQUENCE OF EMBRYO AND ENDOSPERM IN APOMICTIC AND SEXUAL FORMS OF POA PRATENSIS[1]

ETLAR L. NIELSEN

Introduction

It is well established that the proembryo may consist of several cells before the first division of the fusion nucleus in apomictic forms of Kentucky bluegrass, *Poa pratensis* L. The significance of some of the figures of the developing female gametophyte of this species published by ANDERSON (3) has only recently been recognized. TINNEY (5) found that pollination was unnecessary to induce the formation of proembryos but that without pollination no endosperm was formed. This was recently substantiated by ÅKERBERG (1, 2), whose demonstration of the necessity of fertilization of the polar nucleus for endosperm formation was based upon an extensive series of emasculations. The sizes of the complements of chromosomes in this tissue could be accounted for only by assuming that fertilization had occurred. The latter observation was confirmed by NIELSEN (4).

Opportunity was offered for making some additional observations on this phenomenon during the course of cytological studies of the species. It appears desirable to record briefly some of the more salient facts that have been brought to light.

Material and methods

Kentucky bluegrass plants flower in the early morning at Madison, Wisconsin. Anthers are exserted and the pollen is mostly shed before 6:00 A.M. on bright days of late May.

Material was gathered from known sexual and apomictic plants growing in the breeding nurseries (4). From each of two sexual and two apomictic plants all spikelets of panicles without lowermost florets at anthesis were removed on the first day these plants flowered. The remaining spikelets, those wherein the lowermost florets were at anthesis, constitute the material upon which this study was based. These were fixed in Sax's modification of Navashin's fluid. The first fixation was made at 9:00 A.M., and subsequent ones at 24, 48, 120, 216, and 264 hours later. The material was transferred to 70% alcohol the following day. Dehydration was by the usual alcohols, and the imbedded material was sectioned at 15 μ. Iodine-ripened haematoxylin was used for staining.

Observation was limited to the lowermost floret of the spikelet in compiling the accompanying data, with one exception. Data for florets 20 hours before anthesis were derived from the second floret of the spikelets of the first fixation. This was based upon the assumption that this floret would have been at anthesis the day after fixation had it remained upon the plant.

There is usually considerable delay in the migration of the polar nuclei as well as in the division of the fusion nucleus. Several arbitrary values were therefore set for the different positions of the nuclei of the developing female gametophyte. These are illustrated in

[1] Results of co-operative work between the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station, Madison.

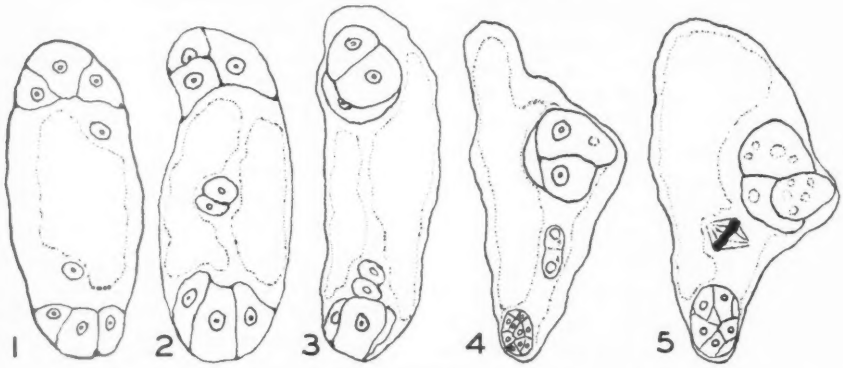
figures 1–5. It is recognized that there may be deviations from these arbitrary classes, but for purposes of tabulation this affords a method of establishing comparative data.

Results

The observations are tabulated in table 1. The developmental sequence is markedly different in the two types of plants during the first 2–3 days following anthesis. A proembryo averaging eight to sixteen cells was present in the apomictic plant a day before anthesis. Only two observations were made at this stage for the sexual plants. The polar nuclei had migrated toward the center of the embryo sac in each case.

Four hours after anthesis the proembryo in embryo sacs of apomictic plants averaged from sixteen to thirty-six cells in size, the polar nuclei being in positions of from 1 to 3. Florets of sexual plants collected at comparable intervals showed varying stages of development ranging from a four-nucleate endosperm (one instance) to stages of fusion of the polar nuclei in a position adjacent to the zygote and proembryo (five instances). In two embryo sacs the polar nuclei were lying in the central position. The proembryos of embryo sacs of the sexual plants averaged about a third the size of those in their apomictic counterparts.

In no instances were endosperm nuclei observed in material taken from the apomictic plants 28 hours after anthesis. Nine of twenty comparable embryo sacs of sexual plants had developed free-nucleate endosperm, the number of nuclei averaging eight and sixteen.



FIGS. 1–5.—Stages of development of embryo sac in *Poa pratensis*. Fig. 1, polar nuclei migrating toward center of embryo sac. Fig. 2, polar nuclei lying near center of embryo sac. Fig. 3, polar nuclei lying adjacent to egg. Fig. 4, polar nuclei migrating toward antipodals. Fig. 5, first division of polar fusion nucleus just below antipodals. Figures are not drawn to scale.

Two days after anthesis the endosperm tissue in the florets of the apomictic plants had developed greatly, the average number of nuclei observed being 116 and 236. The endosperm nuclei of the sexual plants averaged 13 and 16 at the corresponding stage. The proembryos averaged 10 and 12 cells in the same florets and were approximately one-fourth as large as those in the embryo sacs of the apomictic plants with which they are compared.

Five days after anthesis, and subsequently, there is no clear-cut difference between the two types of plants in em-

bryo and endosperm development. The endosperm is partially cellular and the undifferentiated embryo rather large. Differentiation of the latter is well under way at 9 days, as is food storage in the endosperm. Eleven days after anthesis the endosperm is in dough and, to all appearances, the embryo differentiated into its several tissues.

The slow initial growth of the proembryo in florets of the sexual plants during the first 48 hours following anthesis is notable as well as the apparent rapid acceleration during the next 3 days. These

TABLE 1

DEVELOPMENT OF EMBRYO IN RELATION TO THAT OF ENDOSPERM IN SEXUAL AND IN APOMICTIC PLANTS OF *Poa pratensis**

Plant	Time (hours) from anthesis	Florets examined	Character of zygote or proembryo†		Position of polar nuclei					Average number of endosperm nuclei or character of endosperm
			Zygotes observed	No. cells in proembryo	1	2	3	4	5	
	Sexual									
70-11	4	3	.....	11.0	....	2	1	....	....	.....
	28	13	.....	11.7	....	1	....	7	1	8.0
	52	8	.....	10.5	....	....	....	4	....	16.2
	124	16	.....	Massive	....	....	....	....	....	259 to cellular at base
	220	13	.....	Differentiating	....	....	....	....	....	Cellular and food materials stain densely
	268	16	.....	Differentiated	....	....	....	....	....	In dough
72-16	− 20	2	2	.....	....	2	....	....	....	.....
	4	6	.....	6.6	....	....	5	....	....	4.0
	28	7	.....	26.0	....	....	1	1	....	16.2
	52	5	.....	12.5	....	....	1	1	....	13.3
	124	6	.....	44.4	....	....	....	....	....	Free nucleate to cellular
	220	6	.....	Differentiating	....	....	....	....	....	Cellular
	268	16	.....	Differentiated	....	....	....	....	....	In dough
	Apomictic									
72-5	− 20	4	2	8.0	2	1	1	....	....	.....
	4	9	2	36.1	1	4	4	....	....	.....
	28	14	.....	23.7	2	2	3	3	3	.....
	52	10	.....	45.2	....	2	....	....	....	116 to cellular at base
	124	7	.....	85.2	....	....	....	....	1	Cellular at base
	220	7	.....	Differentiating	....	....	....	....	....	±Cellular to cellular
	268	14	.....	Differentiated	....	....	....	....	....	In dough
72-14	− 20	8	.....	14.2	....	6	2	....	....	.....
	4	12	.....	17.4	....	10	2	....	....	.....
	28	2	.....	29.5	....	....	....	....	2	.....
	52	4	.....	36.3	....	....	....	....	1	236 to cellular at base
	124	4	.....	52.0	....	....	....	....	....	±Cellular
	220	18	.....	Differentiating	....	....	....	....	....	Cellular
	268	10	.....	Differentiated	....	....	....	....	....	In dough

* Progenies of plants 72-5 and 72-14 contained 0 and 8.3%, respectively, offtype plants; and those of plants 70-11 and 72-16 were 100% aberrant, no two plants being morphologically alike.

† Zygote includes unicellular members of embryo sacs.

are evident from a comparison of the developmental sequence in sexually and apomictically reproducing types. The late division of the fusion nucleus in the embryo sacs of the apomictic plants is fully in accord with earlier observations. There is published evidence that pollination is necessary for endosperm formation, and, without this being effected, the ovule aborts. There is evidence also from the chromosome numbers present in the endosperm tissue of apomictic plants that fertilization must have taken place to account for the sizes of the complements present. Although this evidence has been established, it is difficult from the present data to arrive at a satisfactory conclusion that accounts for the long delay from the time of pollination to the onset of formation of endosperm in the apomictic plants. The data presented indicate that fertilization to form the polar fusion nucleus could hardly have taken place less than 24 hours following anthesis. Usually it is not prior to this time that the polar nuclei are in intimate association with one another. When these observations are considered with reference to the sequence of events in the sexual plants, it becomes evident that the two types of plants differ also in respects other than those determinable from cytological study. Other techniques than those employed will apparently have to be administered to extensive materials before the underlying causes can be definitely determined.

Summary

The comparative development of the embryos and endosperms was examined in two plants each of known apomictic or sexual reproductive tendencies. The development of the embryo is well under way before the first division of the fusion nucleus in apomictic plants of *Poa pratensis*. In sexual individuals the endosperm development is in advance of that of the zygote or proembryo during the first 2 days following anthesis. Five days after pollination, and subsequently, the development of both embryos and endosperm in the two differently reproducing types of plants is essentially parallel, and the two types are then indistinguishable from each other.

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RESPONSE OF GUAYULE TO ALTERNATING PERIODS OF LOW AND HIGH MOISTURE STRESSES

H. M. BENEDICT, W. L. McRARY, AND M. C. SLATTERY[1]

Introduction

The yield of rubber per acre obtained from guayule is determined by the amount of rubber-bearing tissue present and its rubber content. Results to date (**1, 2, 4, 7, 9, 10**) have indicated that the rubber-bearing tissues are produced during periods of active growth but that their rubber content increases during periods of limited growth caused by drought, low temperatures, and nutrient deficiencies. This raised the question as to which method would yield the most rubber: (*a*) to allow the plants to grow vigorously for short periods of time and then to subject them for short periods to conditions favorable for building-up of rubber but unfavorable for growth, or (*b*) to allow them to grow actively for a long period, followed by a single period of conditions favorable for rubber formation. In other words, was it necessary, to obtain large yields, for the shrub to build up its rubber content as it grew—and, if so, how long should the periods of active or limited growth last—or could a large shrub be produced first which would accumulate rubber later?

This paper presents results obtained at Salinas, California, from studies on growth and carbohydrate, resin, and rubber content of guayule subjected to alternating periods of abundant moisture and drought for different lengths of time. The data, in addition to yielding some information on the above points, also indicate the changes in carbohydrates and resins induced by the various drought conditions.

Methods

Nursery plants seeded in the spring of 1943 were dug the first week in March, 1944, and were carefully selected for uniformity of crown diameter between $\frac{5}{32}$ and $\frac{6}{32}$ inch. All leaves and any stem tissues more than 2 inches above the crown were pruned off. Large wooden boxes 14 × 14 inches square and 20 inches deep were filled to a depth of 3 inches with coarse gravel to allow for drainage and then to within an inch of the top with soil consisting of a mixture of two parts of soil (Chualar loam), one part of leaf mold, and one-half part of sand by volume. The plants were set in these boxes on March 6, 1944, one plant per box. Following a period of 2 months in the greenhouse at 60°–70° F., they were placed outside until September 27. Only 0.62 inch of rain fell during this outdoor period. From the time of return to the greenhouse until the final sampling on May 6, 1945, the plants grew at a temperature between 70° and 80° F.

Four different treatments were applied: (A) plants grown continuously with abundant water (low moisture stress); (B) plants grown for 2 months under low moisture stress followed by 2 months of drought (high moisture stress), with this cycle repeated until the end of the experiment—a total of four wet and three dry periods; (C) plants grown for

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4 months under low moisture stress followed by 4 months under high moisture stress, with this cycle repeated until the close of the experiment; (D) plants grown for 10 months under low moisture stress followed by 4 months under high moisture stress.

The experiment was designed to last 16 months, but, because of the discontinuance of the project, the final harvest was made 2 months ahead of schedule. Thus, the final results on treatment B were obtained at the close of a wet, rather than a dry, period, and those on treatment C were taken after a drought period of 2, instead of 4, months.

The moisture stresses or degrees of soil drought to which the plants were subjected were indicated by the electrical resistance method described by BOUYOUCOS and MICK (3). The plaster of Paris blocks were buried 10 inches from the surface of the soil in several of the boxes. The resistance of the blocks with moisture content of the soil at field capacity was about 300 ohms; at the wilting-point, between 450,000 and 600,000 ohms (5, 8). When the plants were grown in the presence of abundant soil moisture (low moisture stress), it was endeavored to maintain the resistance of the blocks between 300 and 800 ohms, although on occasion the moisture stress became higher and the resistance rose to as high as 3,000 ohms. However, under the latter condition the plants never showed any signs of wilting even in the middle of the day. When the plants were grown under conditions of high moisture stress, the procedure was similar to that recommended by HENDRICKSON and VIEHMEYER (6). The soil was allowed to dry until the resistance of the blocks reached approximately 250,000 ohms. Then enough water was added to reduce the resistance to about 300 ohms (field capacity of the soil), and the soil was again allowed to dry until the resistance reached 250,000 ohms. This cycle was repeated throughout the dry period.

The amount of water required to cause a drop in resistance from 250,000 to approximately 300 ohms was between 9 and 12 liters for each box of soil. The logarithms of the resistances in ohms of the blocks in treatments A, B, and C from November 6 to May 6 are shown in figure 1. The resistance curve in treatment D was the same as in treatment A until January 6, then it equaled that of B and C during their drought periods. The curve of treatment A is the mean of three replications, treatment B of five, and treatment C of two.

Plants from the various treatments were harvested at the end of each period of moisture stress. Thus plants of treatments A and B were harvested every 2 months; of treatment C, every 4 months; and of treatment D, after 10 and 14 months. Five replications of three plants each were harvested on each date. The various determinations were made on each replication. The treatments and replications were arranged in a randomized block design, the randomization scheme being taken from TIPPETS' *Random Sampling Numbers* (12). Analyses of variance were carried out on the data obtained.

In harvesting the plants a steel bar was driven into the soil about 2 or 3 inches from the main tap root. Leverage was then applied so as to lift the plant out of the ground. This is an undesirable way to harvest the roots as the many small rootlets break off and are left behind, and only the main tap roots and the larger lateral roots are obtained. Tests indicated that on a dry-weight basis about 15–20% of all roots present were left behind by this manner of har-

vesting. When the data on the roots are being considered, this source of error must be taken into account. The portions of the plants thus recovered were comparable with those recovered by methods of digging in the field, and the data are thus of value in indicating results that would have been obtained from

of the fresh material in a ventilated dryer at 65° C. for 48 hours. The carbohydrate fractions, free sugars, levulins, and inulin were analyzed according to the method described by TRAUB and SLATTERY (**15**), and pentosans by the method of MCRARY and SLATTERY (**11**). Rubber and resins were determined by

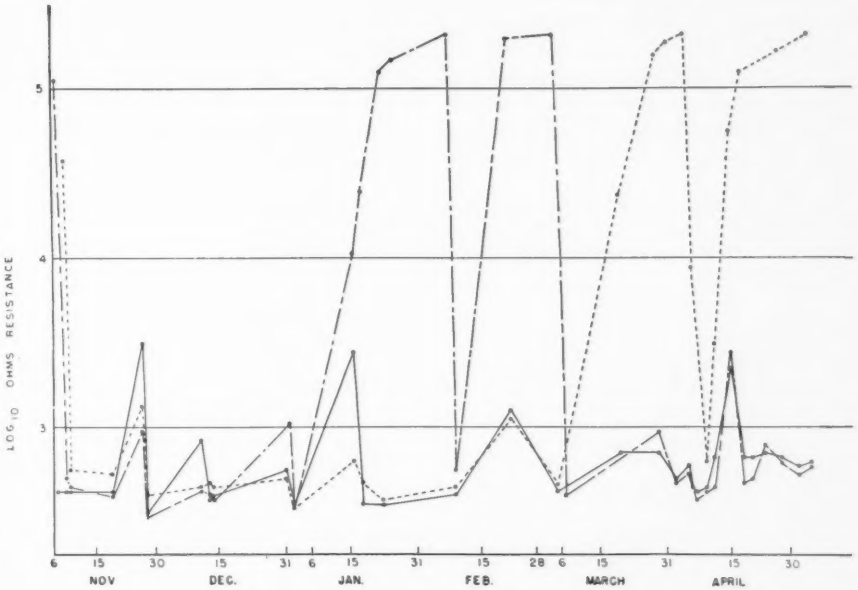

FIG. 1.—Moisture stress in different treatments from November 6 to May 6 expressed as $\log^{10}$ of resistance of Bouyoucos blocks in ohms. Value of 2.48 represents field capacity; 5.65–5.78 the wilting-point. Treatment A, solid line; treatment B, broken line; treatment C, dotted line.

field plantings grown under similar climatic and edaphic conditions.

After the plants were removed, the roots, stems, and leaves were separated. The fresh weight, percentage moisture, and dry weight of these fractions of each replication of each treatment were determined separately. The carbohydrate, rubber, and resin contents of these plants were also determined.

The percentage moisture and dry weights were obtained by drying aliquots

the turbidimetric method described by TRAUB (**13**).

Results

EFFECT ON DRY WEIGHTS

The most pronounced effect of the treatments was found in the weight of the leaves (table 1). High moisture stress caused a reduction in leaf weight. On the addition of soil moisture, the leaf weight never increased to that of plants grown under continuous low moisture stress.

The increases in dry weights of the roots and stems in treatment C and D (see also fig. 2) were limited by high moisture stress but to a lesser degree than those of the leaves. The first dry period limited the increase in weight of the roots and stems of the plants in treatment B. After the first cycle, however, dry weights of the stems at the end of each period of high stress in this treatment were not significantly different from those of treatment A but were significantly lower at the end of the periods of low moisture stress. These results on the stem growth of plants in treatment B cannot be accounted for, but they seem to be related to the proportion of leaves to stem at each change of moisture stress coupled with the relative short duration of the high stress period.

The greatest differences in dry weights were not between treatments but between dates. The proportionate increases were greater during the period from March to September than from September to March regardless of treatment. The enormous increase in dry weight between March and May, 1945,

TABLE 1

DRY WEIGHT PER PLANT (GRAMS) OF GUAYULE GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATION. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Leaves:							
A	2.32	9.71	21.40	29.20	33.49	43.41	70.23
B	2.32	*5.90*	18.41	*15.75*	25.03	*23.49*	54.68
C	2.32	9.71	.....	*15.37*	.....	33.48	*27.43*
D	2.32	.....	.....	.....	33.49	.....	*27.11*
Difference required $P<0.05$	.....	2.19	2.12	2.24	1.85	5.61	4.38
Stems:							
A	1.32	4.27	17.23	23.47	29.12	36.87	63.64
B	1.32	*3.34*	14.30	*23.59*	26.54	*35.01*	54.81
C	1.32	4.27	.....	*20.02*	.....	28.32	*49.97*
D	1.32	.....	.....	.....	29.12	.....	*40.93*
Difference required $P<0.05$	.....	0.66	1.98	3.19	1.78	3.44	5.56
Roots:							
A	1.29	4.08	7.62	9.04	12.50	13.25	18.18
B	1.29	*3.67*	5.54	*8.75*	10.60	*11.07*	17.32
C	1.29	4.08	.....	*8.37*	.....	11.24	*14.04*
D	1.29	.....	.....	.....	12.50	.....	*11.84*
Difference required $P<0.05$	.....	*	0.91	*	1.52	1.69	2.46
Total:							
A	4.93	18.06	46.25	61.71	75.11	93.53	152.05
B	4.93	*12.91*	38.25	*48.09*	62.17	*69.57*	126.81
C	4.93	18.06	.....	*43.76*	.....	73.04	*91.44*
D	4.93	.....	.....	.....	75.11	.....	*79.88*
Difference required $P<0.05$	.....	3.85	4.63	4.19	5.15	5.93	10.40

* Not significant.

is of interest as it occurred in all treatments but not to such an extent in dry treatments as in wet. As the available moisture and temperature were varied over the same ranges during both periods of the year, these results emphasize the importance of some other factor associated with season, such as hours of sunlight or daylength, on the growth of guayule.

At the close of the experiment the plants in treatment A had the greatest amount of rubber-bearing tissue (stems and roots) and those in treatment D the least, with weights of the plants of treatments B and C falling in between (fig. 2).

Carbohydrate content

The percentages of free sugars, levulins, and inulin on the various harvest dates are shown in table 2. Of these water-soluble carbohydrates, only the content of levulins in the stems and roots showed any marked response to the treatments. During periods of high moisture stress the levulin percentage increased; during periods of low moisture stress following a high moisture stress it decreased. The percentages of free sugars and inulin were always small and did not vary consistently with treatment.

To determine whether the reductions

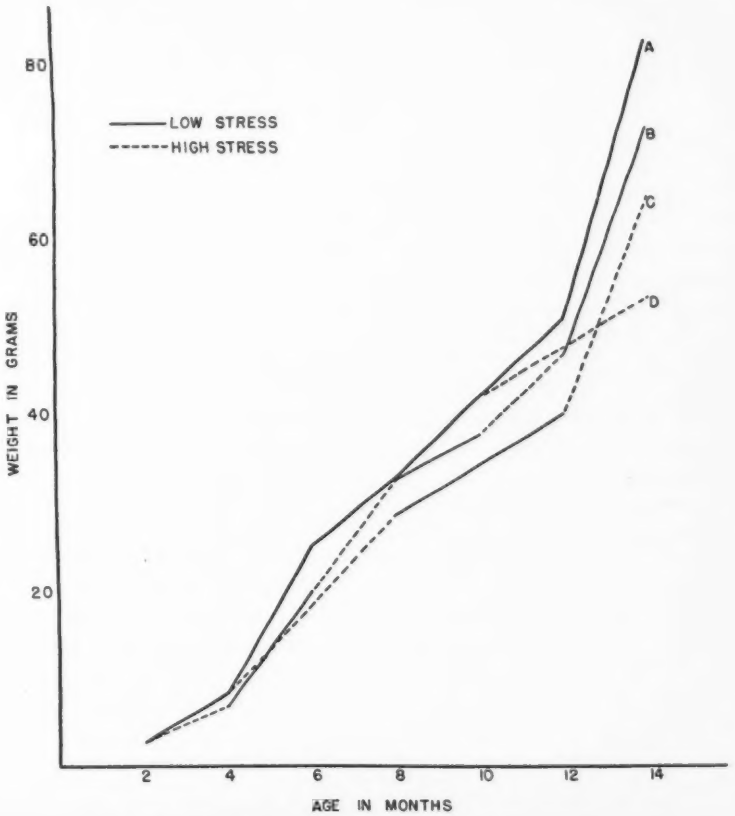

Fig. 2.—Dry weight per plant of stems and roots in different treatments

in percentage of levulins during periods of active growth following periods of slow growth represented a true loss, the grams per plant were calculated for each harvest date. These results, combining the amounts in roots and stems, have been plotted in figure 3, along with the values for free sugars, inulin, and total water-soluble carbohydrates. They show that the absolute levulin content increased markedly under conditions of high moisture stress and that the levulins pre-

TABLE 2

WATER-SOLUBLE-CARBOHYDRATE CONTENT (PERCENTAGE BASIS) OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATION. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
	Free sugars*						
Leaves:							
A	0.48	1.10	0.91	0.41	0.42	0.41	0.65
B	.48	*0.69*	1.01	*.51*	.33	*.43*	.90
C	.48	1.10	.....	*.46*	.....	.37	*.71*
D	.48	.....	.....	.....	*.42*	.....	*.79*
Stems:							
A	.65	0.71	0.39	.36	.39	.30	.37
B	.65	*0.71*	0.41	*.60*	.34	*.55*	.33
C	.65	0.71	.....	*.67*	.....	.26	*.62*
D	.65	.....	.....	.....	*.39*	.....	*.56*
Roots:							
A	.55	0.56	0.29	.34	.31	.37	.49
B	.55	*0.77*	0.34	*.44*	.32	*.51*	.46
C	.55	0.56	.....	*0.47*	.....	0.33	*.42*
D	0.55	.....	.....	.....	*0.31*	.....	*0.41*
	Levulins*						
Leaves:							
A	0.46	0.85	1.42	0.59	0.49	0.48	0.57
B	0.46	*0.69*	1.61	*0.54*	0.48	*0.38*	0.53
C	0.46	0.85	.....	*0.66*	.....	0.40	*0.75*
D	0.46	.....	.....	.....	*0.49*	.....	*0.57*
Stems:							
A	1.30	4.75	3.26	1.91	2.44	1.80	1.26
B	1.30	*7.82*	3.07	*5.25*	1.88	*3.47*	0.89
C	1.30	4.75	.....	*5.60*	.....	1.33	*5.52*
D	1.30	.....	.....	.....	*2.44*	.....	*4.71*
Roots:							
A	2.37	5.24	3.25	2.13	2.56	2.73	1.20
B	2.37	*11.07*	2.95	*5.78*	2.78	*5.85*	0.61
C	2.37	5.24	.....	*6.29*	.....	2.25	*6.14*
D	2.37	.....	.....	.....	*2.56*	.....	*6.61*

* Difference required for significance ($P < 0.05$) between means: free sugars, 0.22; levulins, 0.78; inulin, 0.19.

TABLE 2—*Continued*

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF TRANSPLANTS						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
	Inulin*						
Leaves:							
A	0.05	0.33	0.52	0.42	0.42	0.36	0.41
B	.05	.44	.41	.45	.66	.48	.35
C	.05	.33	.....	.50	.....	.48	.27
D	.05	.....	.....	.....	.42	.....	.42
Stems:							
A	.27	.06	.19	.25	.39	.12	.15
B	.27	.31	.20	.15	.18	.53	.19
C	.27	.06	.....	.52	.....	.26	.40
D	.27	.....	.....	.....	.39	.....	.21
Roots:							
A	.11	.21	.20	.22	.31	.26	.10
B	.11	.28	0.13	.13	.25	.25	.23
C	.11	0.21	.....	0.19	.....	0.19	.25
D	0.11	.....	.....	.....	0.31	.....	0.38

viously stored disappeared when the plants were changed from high to low moisture stress. Such large changes did not occur in content of inulin and free sugars; hence the levulins appear to be the important reserve carbohydrates.

TRAUB and SLATTERY (**16**) have pointed out that the levulins may be fractionated into two major portions, the 89%-ethanol-soluble and the 89%-ethanol-insoluble fractions. Their evidence also indicated that the former were of a much lower molecular weight and hence of lower polymer type than the latter. They also emphasized the importance of determining these two fractions in physiological experiments with guayule. In table 3 are shown the percentages, in the stems and roots of the plants, of the total levulins that are insoluble in 89% ethanol—the fraction with higher molecular weight. With one exception, plants grown under high moisture stress showed a greater proportion of their levulins to be of the higher-molecular-weight type than plants grown under low moisture stress. This one exception (treatment C, May, 1945, harvest) occurred while large quantities of levulins were accumulating (fig. 3*C*). In this instance the levulins may have been forming at such a rapid rate that the low-molecular-weight type was being produced at a greater rate than the high-molecular-weight type. When the moisture stress was changed from low to high, the percentage of the 89%-ethanol-soluble fraction of the levulins decreased; when changed from high to low, it increased. It is interesting that, although periods of high stress induced an increase in the proportion of high-molecular-weight levulins, it had little effect on the inulin content.

Under the conditions of the experiment there was no correlation between the moisture stress under which the plants were growing and the percentage of pentosans (table 4). The percentages increased to a maximum about the November 6 or the January 6 harvest and

then decreased to the end of the experiment. Thus, in contrast to the so-called "water-soluble carbohydrates" (table 2), the pentosan content was more affected by season of the year than by moisture stress.

RESIN CONTENT

The resin percentage was not affected greatly or in any consistent manner by moisture stress but did vary seasonally (table 5). It generally increased in leaves and stems up to the September and November harvests, respectively, and then decreased until the May 6 harvest. In the roots the percentage gradually decreased from the start to the close of the experiment.

The grams of resins per plant (stems and roots combined) on the various harvest dates increased steadily up to the

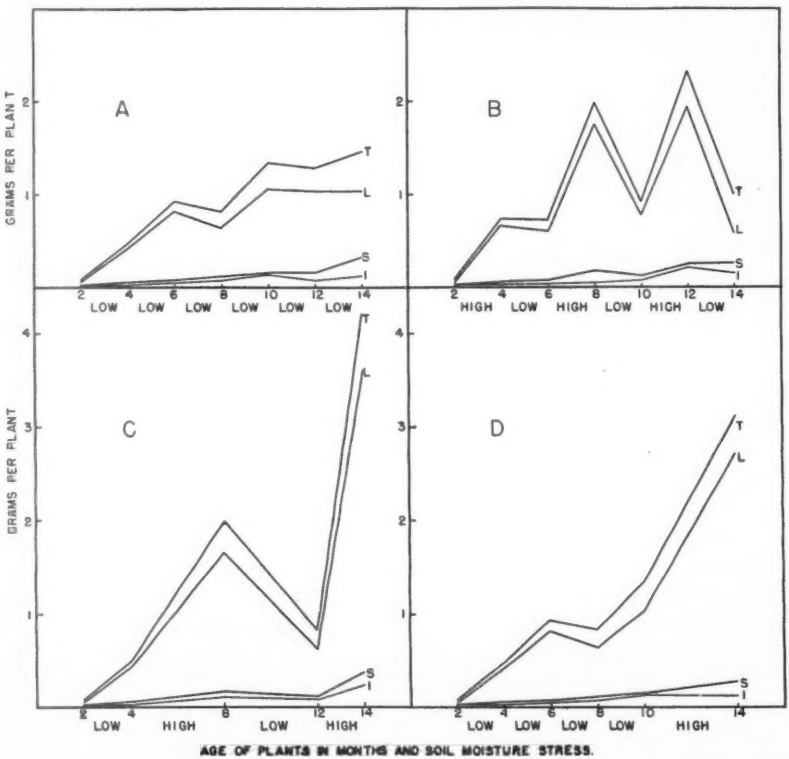

FIG. 3.—Grams of free sugars (S), levulins (L), inulin (I), and total (T) per plant in different treatments at indicated ages following periods of low or high soil moisture stresses.

Of the carbohydrates determined, the levulins were the most important reserve compounds. TRAUB (14) has shown that the pentosans may serve as reserve compounds under severe conditions, but it would seem that the conditions in these experiments did not induce their utilization in this manner.

November 6 harvest (fig. 4). Between November 6 and January 6, plants of treatments A, B, and D actually lost resins, but after January 6 they continued to gain in resins until the final harvest. The loss cannot be accounted for, but, because it occurred in all treatments, it is suggested that the season of the year from November 6 to January 6 is unfavorable for the formation of resins in guayule plants grown under these conditions of temperature and moisture in the greenhouse.

TABLE 3

PERCENTAGE OF LEVULINS INSOLUBLE IN 89% ETHANOL IN PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATION. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

Plant part and treatment	Date of harvest and age of transplants*					
	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Stems:						
A	53.7	42.9	45.6	25.0	33.3	19.1
B	*65.0*	30.9	*58.1*	40.0	*52.4*	19.1
C	53.7	.....	*60.9*	.....	29.4	*34.4*
D	53.7	42.9	45.6	25.0	.....	*34.9*
Roots:						
A	38.4	37.2	34.7	20.4	35.9	30.8
B	*62.3*	33.9	*46.4*	30.9	*50.3*	41.0
C	38.4	.....	*53.1*	.....	34.3	*18.2*
D	38.4	37.2	34.7	20.4	.....	*47.8*

* Difference required at $P < 0.05$: between means, 10.1%; for interaction, 14.3%.

RUBBER CONTENT

The rubber content in the leaves was always less than 1% (table 6). The only significant change that occurred in the leaves in all four treatments was the marked increase in both percentage and grams per plant between the last two harvest dates.

During the first 8 months of the experiment or up to the November 6 harvest, the percentage of rubber in the roots and stems of plants growing under high moisture stress increased more, or decreased less, than the percentage in plants under low moisture stress. The grams of rubber per plant during this time increased more in the plants under high moisture stress than in those under low moisture stress (fig. 4).

From the November 6 harvest until and including the March 6 harvest, the percentage of rubber in the roots and stems in all treatments decreased, regardless of moisture stress. This decrease in percentage was greater in plants grown with low moisture stress following high moisture stress (treatment C) than in plants under continuous low moisture stress (treatment A). During this same period there was only a slight increase in grams of rubber in the roots and stems of plants grown under either high or low moisture stress.

Except for stems in treatment B, the percentage of rubber in the roots and stems of all treatments increased markedly from March 6, 1945, to May 6, 1945. The greatest increase was in plants of

TABLE 4

PENTOSAN CONTENT OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATIONS. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF PLANTS*						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
	Per cent						
Leaves:							
A	2.13	2.72	3.33	2.79	2.13	1.58	1.69
B	2.13	*3.20*	3.13	*3.09*	2.00	*1.88*	1.97
C	2.13	2.72	.....	*3.27*	.....	1.72	*1.95*
D	2.13	.....	.....	.....	2.13	.....	*2.04*
Stems:							
A	2.13	2.46	2.86	2.54	2.42	2.37	1.97
B	2.13	*2.42*	2.64	*2.72*	2.17	*2.19*	1.82
C	2.13	2.46	.....	*2.68*	.....	2.45	*1.77*
D	2.13	.....	.....	.....	2.45	.....	*2.07*
Roots:							
A	1.35	1.43	1.37	1.19	1.49	1.26	1.06
B	1.35	*1.32*	1.39	*1.32*	1.53	*1.23*	1.10
C	1.35	1.43	.....	*1.36*	.....	1.31	*1.10*
D	1.35	.....	.....	.....	1.49	.....	*1.22*
	Grams per plant						
Leaves:							
A	0.049	0.264	0.712	0.815	0.713	0.686	1.187
B	.049	*.189*	.576	*.581*	.501	*.442*	1.077
C	.049	.264	.....	*.503*	.....	.576	*0.535*
D	.049	.....	.....	.....	.713	.....	*0.553*
Stems:							
A	.028	.105	.493	.596	.705	.874	1.254
B	.028	*.081*	.378	*.642*	.576	*.767*	0.998
C	.028	.105	.....	*.537*	.....	.694	*0.884*
D	.028	.....	.....	.....	.705	.....	*0.847*
Roots:							
A	.017	.058	.104	.108	.186	.167	0.193
B	.017	*.048*	0.077	*.116*	.162	*.136*	0.191
C	.017	0.058	.....	*0.114*	.....	0.147	*0.154*
D	0.017	.....	.....	.....	0.186	.....	*0.144*

* Difference required for significance ($P < 0.05$): between means of percentages, 0.35; between means of grams per plant, 0.206 for leaves, 0.183 for stems, and 0.35 for roots from November 6 harvest to end of experiment.

treatment C, which were growing under high moisture stress following a period of low moisture stress. The least increase—or a decrease—occurred in the plants of treatment B that were growing under low moisture stress following high moisture stress. The percentage of rubber in treatment D increased from January 6 to May 6. The grams of rubber per plant in the roots and stems increased markedly tion, the grams of rubber in the plants grown under continuous low moisture stress (*RU-L*) are shown in sections *B*, *C*, and *D*. The progressively larger amounts of rubber in the plants at the end of each period of high moisture stress above that of the plants grown under continuous low moisture stress (treatment B, March harvest, excepted) are clearly shown. The highest yield ob-

TABLE 5

RESIN CONTENT (PERCENTAGE) OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATIONS. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

PLANT PART AND TREATMENT	DATE OF HARVEST AND AGE OF PLANTS*						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Leaves:							
A	3.42	5.72	6.63	4.40	3.31	4.43	4.99
B	3.42	*5.48*	5.61	*4.64*	*3.53*	*3.15*	5.79
C	3.42	5.72	.....	*5.38*	.....	3.87	*4.95*
D	3.42	.....	.....	.....	3.31	.....	*4.72*
Stems:							
A	4.27	5.13	4.70	6.34	4.54	4.46	4.39
B	4.27	*5.77*	5.13	*6.27*	3.94	*3.43*	3.09
C	4.27	5.13	.....	*6.89*	.....	4.57	*4.30*
D	4.27	.....	.....	.....	4.54	.....	*4.84*
Roots:							
A	6.05	5.69	4.03	5.50	3.13	3.17	2.65
B	6.05	*6.02*	4.13	*5.44*	3.80	*3.57*	2.86
C	6.05	5.69	.....	*5.64*	.....	4.04	*3.81*
D	6.05	.....	.....	.....	3.13	.....	*3.69*

* Difference required between means at $P < 0.05$ is 0.48%.

in all treatments in the last 2 months of the experiment. The increase was greatest in treatment C. An analysis of variance showed a highly significant interaction between the contents on March 6 and May 6 between treatments A and C. This indicated that the rate of accumulation of rubber was higher in treatment C than in treatment A.

The grams of rubber per plant in roots and stems are shown in figure 4 for the different treatments (*RU*), and, in addi- tained was from treatment C. Its final yield was 2.247 grams per plant as compared with 1.413 grams per plant in treatment A, a difference of about 59%. At the end of 8 months, treatment C had also yielded the most rubber per plant. At the close of the experiment, treatment D had produced barely significantly more rubber than treatment A. During the early part of the experiment, treatment B showed more rubber per plant at the end of periods of high moisture stress

than treatment A, but at the end of the experiment there was no difference between them.

Discussion

The yield of rubber per plant is the product of the growth of the plant (dry weight) and of the proportion (percentage) of this weight that is made up of rubber. The results obtained in this experiment demonstrate two interacting conditions that affect these two determinants of rubber yield. These are moisture stress and some condition other than temperature associated with the season of the year, such as length of day, light intensity, or hours of sunlight. High moisture stress tended to retard growth, although the amount of retardation varied with the season. Thus, regardless of moisture stress, the plants in all treatments showed the highest rate of growth between March and May, 1945, and the lowest rate during the months just preceding (fig. 2). High moisture stress, except for the period from November 6 to March 6, tended to cause an increase in the proportion of rubber. This increase more than offset the decrease in growth rate, so that more

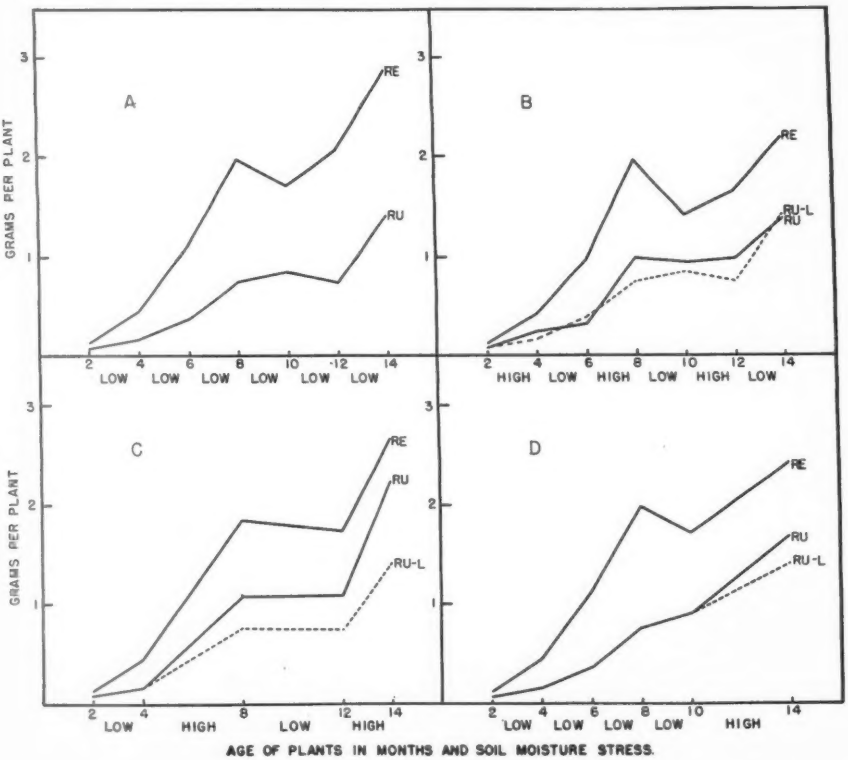

FIG. 4.—Grams of resin (*RE*); rubber (*RU*); and rubber in treatment A (*RU-L*), in roots and stems of plants in different treatments following growth under conditions of low and high soil moisture stress.

grams of rubber per plant were obtained under conditions of high moisture stress than low moisture stress.

The results show that guayule grown under alternating conditions of low and high moisture stress will contain more grams of rubber per plant than will plants grown continuously with low moisture stress and that the amount of the difference will be enhanced with each successive period of high stress. If the duration of these periods is too short, the increase in proportion of rubber will little more than offset the limitation on dry-weight increase so that the product of dry weight by rubber percentage will only slightly exceed that of plants grown in continuous low moisture stress. It is conceivable, also, that if the periods of high stress were too long, the growth of the plant might be so limited that the increased rubber content would not compensate the relative loss in dry weight in affecting the yield of rubber per plant. Under the conditions of these experiments, a 2-month high-stress period was too short and a 4-month high-stress period resulted in a much higher rubber yield under as nearly identical conditions as could be obtained (fig. 4, at age of 8 months). Under other conditions, or with older plants, stress periods of different duration would probably be more desirable. The ideal for which to strive would be a high-stress duration which would induce the maximum increase in proportion of rubber with minimum effect on the increase of dry weight.

If periods of high moisture stress are to be used practically in increasing the yield of rubber, the results emphasize the importance of the time of year in applying the high-stress treatments. During the period from November 6 to

TABLE 6

RUBBER CONTENT (PERCENTAGE) OF PLANTS GROWN UNDER LOW AND HIGH SOIL MOISTURE STRESSES IN CYCLES OF DIFFERENT DURATIONS. (VALUES IN ITALICS OBTAINED AT END OF HIGH STRESS PERIODS)

Plant part and treatment	Date of harvest and age of plants*						
	5/6/44 2 mo.	7/6/44 4 mo.	9/6/44 6 mo.	11/6/44 8 mo.	1/6/45 10 mo.	3/6/45 12 mo.	5/6/45 14 mo.
Leaves:							
A	0.31	0.35	0.39	0.42	0.34	0.18	0.64
B	0.31	*0.38*	0.52	*0.43*	0.30	*0.35*	0.47
C	0.31	0.35	.....	*0.44*	.....	0.29	*0.85*
D	0.31	.....	.....	.....	0.34	.....	*0.86*
Stems:							
A	2.52	0.41	1.25	2.16	1.84	1.50	1.70
B	2.52	*1.48*	1.18	*2.79*	2.25	*2.15*	1.79
C	2.52	0.41	.....	*3.56*	.....	2.83	*3.37*
D	2.52	.....	.....	.....	1.84	.....	*2.74*
Roots:							
A	3.32	3.64	2.02	2.65	2.50	1.40	1.82
B	3.32	*5.23*	2.69	*3.82*	3.35	*2.16*	2.37
C	3.32	3.64	.....	*4.48*	.....	2.58	*4.02*
D	3.32	.....	.....	.....	2.50	.....	*3.51*

* Difference required for significance ($P < 0.05$) between means: 0.13 for leaves, 0.65 for stems and roots.

March 6, conditions in this experiment were unfavorable to the formation of rubber, and periods of high stress did not induce an increase in rubber content. The most favorable time appears to be the spring, when conditions favored both growth and the accumulation of rubber. Plants subjected to high moisture stress during this period practically doubled their absolute rubber content in 2 months (treatment C [fig. 4]).

Because of the seasonal effect, unknown before the experiment was started, the results offer little information as to whether rubber accumulates in a full-grown plant or must be formed as the plant develops. They seem to indicate that, for highest rubber yield, the rubber should be accumulated in the plants as they grow, since the plants of treatment C had a higher rubber percentage and a greater absolute rubber content than those of treatment D. However, the plants of treatment D began and underwent the first half of their dry period during the season when no measurable amount of rubber was formed in any of the treatments. It may be that the rubber percentages in the plants in those two treatments would have differed less if their final dry periods had started and ended at the same time. The comparison of grams of rubber per plant in treatments B and C was made after 8 months instead of at the end of the experiment, because treatment B ended after a low-stress period and treatment C after a high-stress period.

Milled rubber from guayule contains as high as 20% of resin as an impurity. Any treatment which would reduce the resin content of the plant and increase its rubber hydrocarbon content would be highly desirable. Howevef, in these experiments the grams per plant and percentage of resin varied in much the same manner as the rubber hydrocarbon in response to the various treatments.

Although season of the year influenced the amount of change in rubber and resin content of the plants brought about by conditions of high and low moisture stress, it had little effect on the changes in the carbohydrate content in response to these conditions. Thus the free-sugar, inulin, and pentosan content showed no marked response to changes in soil moisture stress at any season of the year. On the other hand, the levulin content always increased during periods of high moisture stress and decreased during the following periods of low moisture stress. This again points to the importance of the levulins as reserve carbohydrates.

Summary

1. Guayule nursery-transplants were grown for 14 months in alternating periods of low and high moisture stress of different durations. The dry weights and the carbohydrate, resin, and rubber contents of the plants were determined at the end of each period of moisture stress. During periods of high moisture stress, the rate of increase in dry weight of the entire plant was depressed, but most of this could be accounted for by the effect on leaf weight. The percentages of levulins, in roots and stems especially, during these periods increased markedly, while the percentages of the other carbohydrates—sugars, inulin, and pentosans—remained fairly constant. Except for the period from November 6 to March 6, the percentages of rubber increased during periods of high stress at a more rapid rate than during periods of low stress.

2. During periods of low stress following high stress, the percentage and absolute content of levulins per plant decreased. This indicated that the levulins

were serving as the principal reserve compounds, inasmuch as the amount of the other carbohydrates did not change greatly.

3. The rubber content on a grams-per-plant basis generally increased throughout the experiment, except for the period November 6–March 6, when it did not change. With this exception, the increase was greater during periods of high moisture stress than during low moisture stress. The absolute rubber content per plant was further increased, above that of plants continuously on low moisture stress, after each successive period of high moisture stress.

4. The results indicate that rubber accumulation can be forced in guayule plants by alternating periods of low and high moisture stress but that the periods of high stress must not be too short and, to induce maximum yields, should occur during seasons of the year other than the winter months.

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EFFECTS OF FRUIT THINNING ON ALMOND FRUITS AND SEEDS

GEORGE A. BAKER[1] AND REID M. BROOKS[2]

Introduction

In 1939 BROOKS (1) initiated studies on growth of the fruit and seed of the almond; growth was presented as averages of measurements at uniform time intervals on the linear dimensions of various parts of fruits and seeds of the Nonpareil and Texas varieties. The present study will indicate the effects of thinning for Nonpareil on the averages and variances (as functions of time) of a linear-dimension, fruit-cheek diameter; on the means, variances, and correlations for three variables, namely, final fruit-cheek diameter, seed-cheek diameter, and seed weight; on the yield of seed per 100 leaves; and on the weight of new terminal wood per 100 leaves.

Material and methods

A mature, healthy, bearing tree of Nonpareil was selected in the almond-variety collection of the University of California at Davis; a fundamental assumption (3) was made that each limb (2 inches in diameter or larger) behaves as a unit independent of adjacent limbs. On March 21, 1944, on this tree, 335 fruits were tagged and their cheek diameters measured. They were selected by inspection as the largest per spur (average cheek diameter of 6.942 mm.) and were well scattered among the lower branches of the tree; these almonds are referred to as "unthinned." On the same day a branch about 2 inches in diameter on the southeast side was thinned to the one largest fruit per spur, all removed fruits being saved and measured. The total, including those left on the branch, was 2766 fruits, with an average cheek diameter of 4.984 mm. and with a standard deviation of 1.119 mm. The lot of 350 tagged fruits left on the branch is called the "first thinning" and had an average cheek diameter of 6.908 mm.

On March 22, 1944, another branch, about $4\frac{1}{2}$ inches in diameter, on the opposite side of the tree was selected and thinned more severely, the largest fruit per spur again being selected with each selected fruit at least 6 inches from its closest neighbor. The removed fruits were saved and measured. In all there were 1623 fruits (including those left on the branch) with an average cheek diameter of 5.360 mm. and with a standard deviation of 1.110 mm. The lot of 188 tagged fruits remaining on the limb is known as the "second thinning" and had an average cheek diameter of 7.149 mm.

On May 16 and 23, 1944, all fruits remaining on a $1\frac{1}{2}$-inch limb near the first thinned limb were tagged and measured. Some of these 65 fruits had been measured throughout the season. This lot is the "unthinned $1\frac{1}{2}$-inch branch."

This variety was in full bloom on March 4, 1944. At the time of thinning the jackets (floral envelopes) had split on the majority of fruits. None of the jackets had fallen, but all were removed before measurements were made.

One can understand the difference in thinning more precisely by observing that on May 11 the number of leaves per original tagged fruit was eighteen for the first thinning and fifty-eight for the second thinning. The cheek diameters of these fruits were measured again on

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March 28; April 4, 11, 18, 25; and May 2, 9, 16, 23. On August 8 the fruits were mature and were harvested; weights of seeds on a comparable moisture basis were recorded on August 17–19. The weight of wet new terminal wood on each branch included in the experiment was determined at harvest time by clipping and weighing the current-season growth in length.

The standard deviation of an individual measurement on cheek diameter of a fruit was estimated from the last two sets of measurements to be 0.23 mm.

Results and discussion

Effect of thinning on growth and dispersion of fruit-cheek diameter

It is to be expected that thinning will result in increased relative size of fruits.

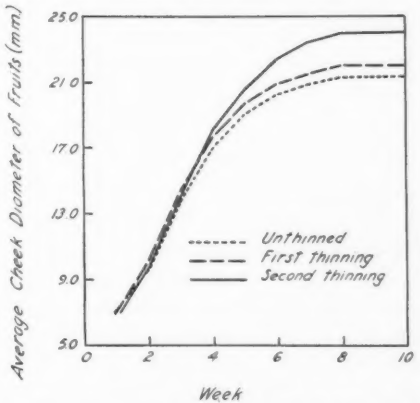

FIG. 1.—Average cheek diameters at weekly intervals for Nonpareil almonds for three degrees of thinning, Davis, California, 1944.

Figure 1 shows the extent to which this rule held true for fruits that remained on the tree until harvest. From the values of averages and variances (figs. 1 and 2) it follows that the averages of the fruit-cheek diameters of unthinned and first thinning did not differ significantly at the beginning of the period (March 21, 1944). The average for the second thinning was slightly higher than for the other two categories; but the measurements were made the next day, March 22—a fact that probably accounts for the difference. At the end of the season the

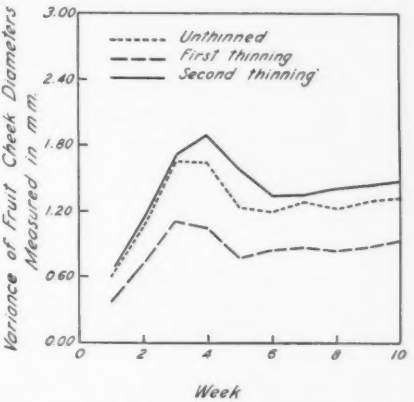

FIG. 2.—Variances at weekly intervals of the cheek diameters of Nonpareil almonds for three degrees of thinning, Davis, California, 1944.

averages of the fruit-cheek diameters of all categories were statistically significantly different.

The effect of thinning on the variances of the cheek diameters of fruits that remained on the tree until harvest seems to be less consistent. The variance curves (fig. 2) for the different categories are strikingly parallel throughout the season, with distinct peaks in midseason. In all cases the variances increase, reach a maximum value, decrease, reach a relative minimum value, and then increase slightly. These features of the variance curves probably reflect the nonsynchronism of the growth curves of individual almond fruits.

At the beginning of the season the variances for both the unthinned and the second thinning were greater than the variance of the first thinning. At the

end of the season the variance for the second thinning is significantly greater than for the first thinning ($P < 0.01$); the variances for the unthinned and second thinning are not significantly different; the variances for unthinned and first thinned are significantly different ($P < 0.05$). These differences in variances do not result from differences in methods of selection, since the largest fruit per spur was selected in each case. In general, one would expect the unthinned fruits to show as great variation as any of the other categories, or greater, since these fruits are a sample from a population subject to a wider range of conditions. The first-thinning fruits were very uniform as compared with the others. Once the selection was made, the relative positions of the categories with respect to size of variance did not change throughout the season. Thinning seems not to be effective in changing the relative variability of the cheek diameter of fruits in the several categories. Apparently, however, its effect on seeds is consistent and marked.

TABLE 1

NUMBERS OF MEASURABLE SEEDS, AVERAGE SEED WEIGHTS, AVERAGE SEED-CHEEK DIAMETERS, AND VARIANCES FOR NONPAREIL ALMONDS FOR DIFFERENT DEGREES OF THINNING, DAVIS, 1944

Degree of thinning	No. of seeds	Average seed weight (gm.)	Variance of seed weight	Average seed cheek (mm.)	Variance of seed cheek
Unthinned 1½-inch branch	37	1.0710	0.00835	8.03	0.3909
Unthinned	115	1.0552	.01582	7.84	.3509
First thinning	250	1.1331	.01089	7.77	.2453
Second thinning	153	1.2495	0.00971	7.48	0.2075

MEANS, VARIANCES, AND CORRELATIONS FOR SEED WEIGHT, SEED-CHEEK DIAMETER, AND FINAL FRUIT-CHEEK DIAMETER

The average weights of seeds for the unthinned, first thinning, and second thinning are highly significantly different and are in the order named for increasing weight (table 1). The variance of the unthinned seed weights is greater than those of the first thinning ($P < 0.05$) and second thinning ($P < 0.01$). The variance of the first-thinning seed weights is not significantly different from that of the second thinning, but the variances do decrease as the degree of thinning increases.

The unthinned do not differ significantly from the first thinning in average seed-cheek diameter. The first thinning and second thinning, also the unthinned and second thinning, are highly significantly different in this measurement. The order for decreasing seed-cheek diameter is unthinned, first thinning, and second thinning. The variance of seed-cheek diameter for unthinned is greater than the variances for the first thinning ($P < 0.05$) and second thinning ($P < 0.01$), but these variances for the first thinning and second thinning do not differ significantly. There is, however, a steady progression downward in the variance of seed-cheek diameters as the degree of thinning increases.

Thinning does not seem to have any striking or consistent effect on correlations between pairs of the following variables: final fruit-cheek diameter, seed-cheek diameter, and cube root of

weight (table 2). The cube root of weight is used in order that the regression equations involving weight may be dimensionally correct. The correlations between final fruit-cheek diameter and cube root of weight of the seed are uniformly high. The correlations between seed-cheek diameter and cube root of weight of seed run fairly uniformly low. Since in the latter two cases a rough approximate standard error of a difference is 0.12, few (if any) of the differences appear significant. A similar standard error for differences in correlation coefficients for final fruit-cheek diameter with cube root of seed weight is 0.05; again, the differences between categories do not appear to be especially significant. Perhaps the coefficient is low for the unthinned. For more exact testing see DAVID (2).

TABLE 2

CORRELATIONS FOR SEED-CHEEK DIAMETER, FRUIT-CHEEK DIAMETER, AND CUBE ROOT OF SEED WEIGHT FOR NONPAREIL ALMONDS FOR DIFFERENT DEGREES OF THINNING, DAVIS, 1944

Degree of thinning	No. of seeds	Seed cheek vs. cube root of weight	Seed cheek vs. fruit cheek	Fruit cheek vs. cube root of weight
Unthinned 1½-inch branch	37	0.27	0.29	0.83
Unthinned	115	.57	.26	.63
First thinning	250	.45	.48	.82
Second thinning	153	0.39	0.23	0.72

In summary, thinning causes a relative increase in weight of seeds and a decrease in variability of the weights. The average seed-cheek diameters are less with increase of degree of thinning. Since, however, the weight increases with degree of thinning, evidently the shape of the almond has changed, becoming longer, broader, or both, but smaller in cheek diameter. These facts partially explain the low correlations between seed-cheek diameter and cube root of seed weight and between seed-cheek diameter and final fruit-cheek diameter. Wrinkling of the seed coats and of the outer portion of the cotyledons may also be a factor. The correlations between final fruit-cheek diameter and cube root of weight of seed run much higher.

That thinning induces a change in shape of almond seeds is borne out by certain observations on peaches. DAVIS (3) noticed that peaches thinned very early had much larger pits; that the seeds were long, with large suture diameters but small cheek diameters; and that the seed coats were wrinkled as compared with those in peaches thinned at a later date. Germination of early-thinned seeds was very low compared with later-thinned ones. These facts raise the question as to how the seed material is stored and in what order. Evidently it is laid down in the middle section of the seed in the plane of the suture and length diameters and gradually fills out as much as possible in the cheek diameter. Thus in early thinning the pits of peaches or the outside (pericarp) dimensions of almonds increase very rapidly early in the season because of the decrease in competition with fruits that normally would stay on relatively late. By the time seed material begins to be stored, the space available for seed growth is large; the lack of leaf surface and of time, as well as competition with wood growth, prevents this space from being completely filled. Al-

though the resulting seeds are heavier, they are thinner in cheek diameter and have wrinkled seed coats.

The unthinned 1½-inch branch almonds did not differ significantly from the unthinned in average weight of seed, average seed-cheek diameter, or variance of seed-cheek diameters. The probability, however, is between 0.01 and 0.05 that the unthinned are more variable in seed weight than the unthinned 1½-inch branch ones. It is important that these two samples can be regarded as random samples from populations with the same mean, since some of the later analysis required information for the unthinned category, which is available only for the 1½-inch branch.

It should be borne in mind in these tests for significant differences that the fruits and seeds in each category may differ from the fruits and seeds in the other categories in both their means and variances.

Effect of thinning on yield of seed

As we have seen, thinning affects the weight of seed. It tends to increase the size of individual seeds; but with increase of thinning there are fewer seeds. We wish to express as accurately as possible the relation between thinning and yield. As a measure of thinning we shall take the number of leaves per harvested fruit (x) and shall express yield in terms of grams of seed per 100 leaves (y). If we plot the logarithms to the base 10 of y (Y) against the logarithm to the base 10 of x (X), we obtain three points that very strikingly lie on a straight line. The linear trend, even though based upon only three points, is statistically very highly significant ($P = 0.0005$). Table 3 gives the details of determining the relation between X and Y.

TABLE 3

LEAVES PER HARVESTED FRUIT (x) AND YIELD OF SEED IN GRAMS PER 100 LEAVES (y) FOR NONPAREIL ALMONDS FOR DIFFERENT DEGREES OF THINNING, DAVIS, 1944

Degree of thinning	x	$X = \log x$	y	$Y = \log y$	Calculated Y
Unthinned 1½-inch branch	17	1.23045	6.399	0.80611	0.80594
First thinning	25	1.39794	4.506	.65379	.65401
Second thinning	70	1.84510	1.772	0.24846	0.24839

$Y = -0.90710X + 1.92208$.

$F = \frac{0.1662}{0.0000000822} = 2{,}022{,}000$ with 2 and 1 degrees of freedom.

$P = 0.0005$.

The corresponding relation between the variables x and y is

$$y = \frac{83.58}{x^{0.90710}}. \qquad (1)$$

If the weights of the individual seeds do not change with degree of thinning, then y would vary inversely as x—that is, $y = k/x$. But since the weight of the individual seeds increases with the degree of thinning, y decreases more slowly than is indicated by $y = k/x$. Thus the difference 1.00000 − 0.90710 measures the ability of the remaining fruits to utilize the increasing leaf surface per fruit. Part of the energy made available by the leaves of a limb goes into the production of new wood as thinning becomes more extreme.

Effect of thinning on growth of new wood

Thinning may be expected to promote the growth of new wood. If we designate the number of leaves per harvested fruit by x (as before) and the grams of new terminal wood per 100 leaves as observed August 10 by w, then we find that

$$w = 0.029333x - 0.47552 . \quad (2)$$

The details of fitting appear in table 4. The trend is again statistically significant ($P = 0.02$), even though we are dealing with only three points. From equation (2) w is zero when $x = 16.2$. Thus, roughly, the weight of new terminal wood per 100 leaves is proportional to the number of leaves per harvested fruit minus the number of leaves per harvested fruit for which there is no new terminal wood growth.

TABLE 4

Leaves per harvested fruit (x) and weight in grams of new wood per 100 leaves (w) for Nonpareil almonds for different degrees of thinning, Davis, 1944

Degree of thinning	x	w	Calculated w
Unthinned 1½-inch branch	17	0.0000	0.0231
First thinning	25	0.2850	0.2578
Second thinning	70	1.5737	1.5778

$w = 0.029333x - 0.47552$.

$F = \frac{1.4049}{0.001290} = 1089$.

$P = 0.02$.

Summary and conclusions

1. Thinning causes an increase in the average weights of the seeds of Nonpareil almond with a decrease in the variability of the weights. This is in contrast with the effect of thinning on variability in fruit-cheek diameter. The cheek diameter of seeds and its variability are less with increased degree of thinning. The result is a distinct change in shape of the almond seeds. That is, heavily thinned almonds are long and broad across the suture diameter and flat or narrow in the cheek diameter. The correlations of final fruit-cheek diameter with seed-cheek diameter and of seed-cheek diameter with the cube root of the seed weight are very low. The correlations of final fruit-cheek diameter with the cube root of seed weight are much higher.

2. When the logarithms of yield of seed in grams per 100 leaves (Y) are plotted against the logarithms of the number of leaves per harvested fruit (X), a very striking straight-line relation results, with the coefficient of X somewhat less than 1 (0.90710). If the number of leaves per fruit did not affect the size of the seeds, then the coefficient of X would be 1.

3. If the weight of new terminal wood in grams per 100 leaves is plotted against the number of leaves per fruit at harvest time, the three observed points lie almost exactly on a straight line. Thus, approximately, the weight of new terminal wood per 100 leaves is proportional to the number of leaves per fruit minus the number of leaves per fruit for which there is no new terminal wood.

4. The results of an investigation on the effect of thinning on the fruits and

seeds of Nonpareil almonds have been presented in detail. A parallel study on the Texas variety in the same season on a closely situated similar tree gave almost identical results. The latter test is not published in detail because it adds nothing new. It does, however, give valuable support to the Nonpareil experiment.

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EFFECT OF DURATION AND INTENSITY OF LIGHT UPON FLOWERING IN SEVERAL VARIETIES AND HYBRIDS OF CASTOR BEAN

N. J. SCULLY[1] AND W. E. DOMINGO[2]

Introduction

In certain populations of castor bean a relatively small percentage of the plants fail to produce flowers, although all are subjected to the same field conditions. Such failure has been recently investigated on a genetical basis by DOMINGO (5), who grew plants throughout the entire growing season at Beltsville, Maryland, and found that the flowerless character was evident only in the segregating generations of certain crosses. In such crosses plants of both the parental and the F_1 populations flowered, and the percentage of flowerless individuals was relatively uniform for the F_2 populations investigated.

Since floral initiation and development in numerous plant species were known to be strikingly controlled by photoperiod, it seemed desirable to determine what effect this factor might have upon the flowering responses of parents, their F_1 progeny, and those F_2 individuals that exhibit the flowerless character.

At Beltsville the growing season for castor bean extends from early May until the middle of October. The length of day from sunrise to sunset increases from $13\frac{1}{2}$ hours on May 1 to $14\frac{9}{10}$ at the solstice, June 21, and then decreases to $11\frac{1}{4}$ hours on October 15. There is some additional twilight both night and morning that may be photoperiodically effective for castor bean, but to what extent is not known. It thus seemed possible, if a photoperiod factor is involved in the flowering of this species, that the critical photoperiod for the flowerless types might be either longer or shorter than natural durations available locally.

Since a flower-inducing stimulus has been transmitted from one variety of soybean to another by grafting, this procedure was also tested for castor beans.

The present study considers the ef-

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fects of photoperiod and grafting upon the flowering of castor-bean plants.

Material and methods

Earlier work (5) had indicated that F_2 populations from crosses between the parent varieties Kentucky 38 and D.P. 227 contained some plants exhibiting the flowerless character. These parents and individuals of their F_1 and F_2 progenies comprised the bulk of the plant material in these experiments. In addition, several domestic varieties or selections were investigated in the initial photoperiodic experiment.

Plants were grown in soil in clay pots or soil beds in the greenhouse, in clay pots in controlled-environment rooms, and in soil beds or field plots in field experiments. Plants grown in the greenhouse received temperatures of about 75° F. and those in the controlled-environment rooms of about 70° F.

Only photoperiods of natural or shorter than natural duration were used in the field. To provide short photoperiods in the field experiments, frames covered with a double thickness of black sateen cloth were placed over the plants at the end of the light period. In the greenhouse, photoperiods of shorter duration than those occurring naturally were obtained by moving the plants into dark chambers at the close of the light period. For longer photoperiods, supplemental incandescent-filament light was used beginning at sunset, either in the greenhouse or in chambers adjacent to it. The intensities of supplemental light varied from 10 to 150 foot-candles.

Plants in the controlled-environment rooms (8) received all their light from a carbon-arc lamp supplemented with incandescent-filament lamps. The intensity of light in these rooms was approximately 2000 foot-candles at pot level.

The place of formation of first flower buds (number of nodes from base of plant) was determined for the various plants rather than the date of opening of the first flowers. This criterion has certain advantages, most important of which is that it is a more accurate measure of the time when flowers were initiated, since it furnishes earlier evidence of initiation and thus shortens the interval during which errors might arise owing to variation among plants in rate of development. It also permits the detection, by dissection, of initiated flowers in certain plants which are killed by frost before blossoming. Plants that were found not to have initiated flowers at the end of the experiment were classed as nonflowering, and the total number of nodes for each was recorded.

Experimentation and results

Experiment I

The purpose of this experiment was to test for any outstanding influence of length of photoperiod upon either the formation of flower primordia or the rate of production of nodes. Seed of nine varieties or hybrid lines of castor bean were planted on August 3, 1944, in 8-inch pots in the greenhouse. Only a limited amount of seed of some of these was available, and, as a result, varying numbers of individuals were present in the different populations. The varieties Doughty 11 and Kentucky 38 were grown in thirty-two pots, and sixteen pots of each were placed on each of two greenhouse trucks. Two other trucks each carried eight pots of the Conner variety and four pots of each of the other six varieties.

Beginning with seedling emergence, one lot containing plants of all varieties was subjected to 8-hour photoperiods of

natural daylight, while a comparable lot received approximately 18-hour photoperiods, the latter consisting of all the natural daylight available plus supplemental Mazda light of about 40 foot-candles intensity from sunset until 12:00 M.

Beginning September 1 and ending September 22, weekly dissections were made of several plants of Kentucky 38 and Doughty 11 varieties on both photoperiods to determine their stage of development. Only plants of Kentucky 38 on the longer photoperiod were found with flower primordia or buds on each of these dates. However, on September 9, varieties 3/12 and 3/31 on both photoperiods each had its first flower clusters macroscopic in size and emerging from the sheaths surrounding the inflorescences. This same stage of development was attained at this time by plants of Kentucky 38 on the long photoperiod. The remaining varieties did not then have macroscopic flower buds.

Since plants of the Kentucky 38 variety were flowering promptly on long but not on short photoperiod, a group of four from short photoperiod were transferred to long photoperiod on September 4. On September 16 all four of these had initiated first flowers above the thirteenth node, while corresponding plants remaining on short photoperiod had produced thirteen nodes and were vegetative.

On October 18 the experiment was terminated, and the node at which the first flower primordia occurred, or the total number of nodes in vegetative plants, was recorded for the remaining plants (table 1).

TABLE 1

EFFECT OF PHOTOPERIOD ON SEVERAL VARIETIES OF CASTOR BEAN IN GREENHOUSE EXPERIMENT I. PLANTED AUGUST 3, 1944. HARVESTED OCTOBER 18, 1944

Variety	8-hour photoperiod				18-hour photoperiod			
	Flowering plants		Vegetative plants		Flowering plants		Vegetative plants	
	No.	Average node at which first flower primordia occurred	No.	Total nodes produced per plant	No.	Average node at which first flower primordia occurred	No.	Total nodes produced per plant
Kentucky 38	0	.....	5	19.0	16	8.4	0	.....
Doughty 11	0	.....	8	19.0	0	.....	8	21.1
Conner	2	12.0	5	17.0	2	17.0	6	17.7
D.P. 58	0	.....	4	17.5	0	.....	4	17.5
D.P. 33	0	.....	4	17.0	0	.....	4	16.5
3/103	0	.....	4	17.5	0	.....	4	17.8
3/31	4	10.8	0	.....	4	11.3	0	.....
3/180	1	10.0	3	17.0	4	13.0	0	.....
3/12	4	8.5	0	.....	4	9.0	0	.....

The most striking result of the different treatments was the flowering response of the Kentucky 38 variety. All plants flowered on the longer photoperiod, but none did so on the shorter one. The average node position of first flower primordia was 8.4 in the former lot as determined at harvest time, while the plants on 8-hour photoperiod were vegetative at harvest and had produced

an average of nineteen nodes. Figure 1 shows one representative plant each from the 8- and the 18-hour photoperiod lots. The response to the longer daylength was obviously prompt, since plants of all varieties had an average of six nodes at the time of thinning when only their cotyledons were expanded.

Responses of the remaining eight varieties were variable. Doughty 11, D.P. 58, D.P. 33, and 3/103 failed to flower on either photoperiod, and the total nodes formed were approximately the same for each of these varieties regardless of treatment. Plants of two other varieties, 3/31 and 3/12, flowered on both photoperiods, and with each the average node position of first flowers was a fraction of a node higher on long than on short photoperiod. Some of the Conner plants flowered on each photoperiod, but the first flowers were at a substantially higher position on the longer one. All four of the 3/180 plants on the longer photoperiod flowered, while only one of four flowered on the shorter one.

FIG. 1.—Castor-bean plants of Kentucky 38 variety grown with different photoperiods: *left*, 8-hour; *right*, approximately 18-hour. (Photographed September 27, 1944.)

EXPERIMENT II

This experiment was designed to determine more accurately the critical photoperiod of variety Kentucky 38. On September 4, 1944, a large planting of seed was made in the greenhouse in 6-inch pots. Prior to germination the latter were divided into six equal lots of sixteen pots each. Five lots were placed on photoperiods of 8, 10, 12, 14, and 16 hours, which consisted of 8 hours of natural daylight plus supplemental incandescent light of 40 foot-candles intensity. The sixth lot was subjected to all the natural daylight available plus supplemental light from about sundown until midnight in order to approximate the long photoperiod in experiment I. This photoperiod was $17\frac{1}{4}$ hours long at the start of experiment II.

On October 3, 1944, two plants from

each lot were dissected, and all were vegetative, with ten nodes each. The remaining plants were still vegetative when dissected on October 30 and averaged thirteen to fourteen nodes.

In flowering response to photoperiod, the results of this experiment were in contrast to those of experiment I.

EXPERIMENT III

This experiment was designed to find why opposite responses to long photoperiod were obtained with variety Kentucky 38 in experiments I and II. Experiment III*A* was concerned with various lengths of photoperiod consisting of natural light supplemented with different intensities of artificial light; experiment III*B*, with effects of long photoperiods with artificial light of high intensity in a plant-growth chamber; and experiment III*C*, with effects of long and short photoperiods on plants grown in midwinter, when total natural radiation was less than at other seasons of the year.

EXPERIMENT III*A*.—Seed was planted in the greenhouse in 6-inch pots on September 9, 1944. The population was divided into eight lots of twenty-two plants each. One lot each was placed on 8-, 10-, and 24-hour photoperiods. Each of these photoperiods comprised 8 hours of natural daylight, and in the case of the two longer ones the remaining light period consisted of incandescent light of 25 foot-candles intensity. Four other lots were placed on 16-hour photoperiods consisting of 10 hours of natural daylight plus 6 hours of incandescent light at intensities of 10, 25, 50, or 150 foot-candles. The eighth lot was subjected to a photoperiod comprising all natural daylight available plus incandescent light of 25 foot-candles intensity from sunset to 12:00 M. This photoperiod was 16 hours and 56 minutes long when the plants of the various lots were thinned on October 11, 1944.

On October 27, 1944, half of the plants receiving 24-hour photoperiod were dissected. All were vegetative, and each had nine nodes. On November 18, 1944, the remaining plants on 24-hour photoperiod were all found to be vegetative, with an average of thirteen nodes. The other treatments were continued until December 18, 1944, at which time all lots were still vegetative. Although the average number of nodes produced by the various lots were quite similar, certain of them differed by amounts which are statistically significant (table 2). Plants on 16-hour photoperiod with supplemental light of 150 foot-candles intensity produced significantly more nodes than those in four other lots receiving either lower intensity of supplemental light or a shorter photoperiod.

No Kentucky 38 plants flowered when subjected to long photoperiods during the fall and winter months in experiments II and III*A*. Accordingly, it seemed desirable to determine whether flowering would occur in this variety if maintained on long photoperiod of a continuous and relatively high intensity such as is available in the controlled-environment rooms at Beltsville (**8**).

EXPERIMENT III*B*.—On November 28, 1944, seed of Kentucky 38 was planted in 4-inch pots. The plants were kept on 8-hour natural photoperiod in the greenhouse until December 8, at which time the cotyledons were expanded on all but a few plants. Twenty-eight of them were then moved to a controlled-environment room at 70° F. and initially with 15-hour photoperiod. Since the room was being used for additional experiments, a longer photoperiod was not attainable. On December 17, 1944, the photoperiod was changed for other

purposes to 18 hours and again back to 15 hours on January 2, 1945.

On January 26 the plants were dissected and ten had initiated flower primordia at the average position of node 8.6. The other eighteen were vegetative and averaged 11.1 nodes per plant. The position of first flowers was only 0.2 node higher than for the same variety in experiment I. However, since only ten of twenty-eight plants flowered as against all plants flowering in the earlier experiment, a valid comparison could not be made.

The purpose of experiment IIIC was to observe whether plants of this variety would remain vegetative on relatively long photoperiods at this time of year. Such long photoperiods would contain a substantially shorter duration of natural daylight than would those employed during August to October in experiment I.

After growth until March 1, 1945, dissection showed that all plants were vegetative in all lots. Those which had been on 8-hour photoperiods averaged 10.9, 9.8, 8.8, and 8.0 nodes, respectively, for the four successive planting dates, while the series on long photoperiods averaged 11.2, 10.1, 9.1, and 8.0 nodes.

TABLE 2

EFFECT OF PHOTOPERIOD AND INTENSITY OF SUPPLEMENTAL LIGHT ON DEVELOPMENT OF KENTUCKY 38 VARIETY CASTOR BEAN IN GREENHOUSE. EXPERIMENT IIIA. PLANTED SEPTEMBER 29, 1944. HARVESTED DECEMBER 18, 1944

Photoperiod (hours)	Natural daylight (hours)	Supplemental light		No. of plants	No. of nodes per plant
		Duration (hours)	Intensity (foot-candles)		
8	8	0	.....	21	14.9±0.1*
10	8	2	25	21	15.5±0.1
16	10	6	150	20	16.0±0.1
16	10	6	50	20	15.7±0.2
16	10	6	25	22	14.9±0.1*
16	10	6	10	22	14.8±0.2*
16†	†	†	25	21	15.0±0.1*

* Significantly different from 16-hour, 150 foot-candles lot, at the 1% level.
† This photoperiod consisted of all natural daylight plus supplemental light from sunset to midnight.

EXPERIMENT IIIC.—Beginning December 27, 1944, four successive weekly plantings of Kentucky 38 seed were made in 4-inch pots. There were two lots of twenty plants each on each planting date, which were placed on short and long photoperiods. The short photoperiod consisted of 8 hours of natural daylight. The long one comprised all natural daylight plus Mazda light of 25 foot-candles intensity until 12:00 M. and varied from 16 hours at the start to 16½ hours at the end of the experiment.

EXPERIMENT IV

It was noted earlier that F_2 populations from crosses between varieties Kentucky 38 and D.P. 227 contained plants that failed to flower when grown in the field at Beltsville. DOMINGO (5) found that a large percentage of the plants formed their first flower primordia early in the season at a relatively low

node position but that a few formed them late in the fall, and therefore at a very high node position, and that about 10% remained vegetative throughout the entire growing season.

Experiment IV was mainly designed to determine what effects a longer growing season and varied photoperiod treatments would have upon the flowering responses of the parental varieties, Kentucky 38 and D.P. 227, and of their F_1 and F_2 progenies and to study in more detail the flowering behavior of Kentucky 38 plants grown with long photoperiods during late winter and spring months.

EXPERIMENT IV*A*—On February 1, 1945, seed of both parents, the F_1, and six F_2 populations was planted in 8-inch pots in the greenhouse. The nine different populations were grouped separately and, following germination, were subjected to a $14\frac{1}{2}$-hour photoperiod consisting of all natural daylight plus incandescent light applied both before and after the natural day. The supplemental light period was regularly shortened as the length of natural day increased. The $14\frac{1}{2}$-hour photoperiod was selected, since it closely approximated the natural photoperiod to which castor beans were subjected following emergence in the field in May. Supplementary light was used in the greenhouse until May 6, when the natural daylength was longer than $14\frac{1}{2}$ hours.

In the various populations the plants that produced visible flower buds were harvested, and the position of first flowers recorded. By May 23 all plants of the parental and F_1 populations as well as 436 of the original 546 F_2 plants had produced flowers.

The 110 F_2 plants that had not produced visible flowers by May 23 were divided into four lots. Three of the lots contained 18 plants each, including individuals from all six F_2 populations, and received photoperiods of 8 hours, 18 hours, and natural daylength in the greenhouse. The 8-hour photoperiod comprised only natural daylight; the 18-hour one consisted of all the natural daylight plus Mazda light of 150 foot-candles for the remaining hours of the photoperiod. The fourth lot, of 56 plants, was planted in the field and subjected to natural daylength. The 18-hour and natural-daylength lots in the greenhouse were grown in soil beds, while the 8-hour lot was grown in 12-inch pots. By June 21, 1945, 34 of the 110 plants had produced macroscopic flower buds. These 34 plants were distributed in approximately equal proportions among the groups on the different photoperiods. The position of the first flower buds for these 34 plants averaged only two to three nodes higher than in the last lot of F_2 plants dissected on May 23. Since the terminal bud of castor bean includes an average of five to six nodes, the flower buds observed on June 21 must have been present as flower primordia on May 23 or earlier and thus were evidently initiated before the differential photoperiod treatments were begun.

A total of seventy-six F_2 plants remained in the four different lots after June 21. None was observed to develop macroscopic flowers prior to September 17, when they were harvested and dissected. On dissection twenty-one plants were found to have microscopic flowers. Fifteen were from the natural-daylength lot in the field and the other six from the 8-hour lot in the greenhouse. These data suggest that the differential photoperiod treatments had no outstanding effects on flowering of F_2 individuals.

Six of the plants that were flowerless at the September 17 dissection were transplanted on October 9 to a greenhouse. They were maintained on a 17-

hour photoperiod until March 20, 1946, without forming flower buds or primordia, although they produced an average of fifty-four nodes each during that period. Apparently such F_2 plants when grown in the field locally are vegetative at time of frost for reasons other than need of a longer growing season.

The flowering data for the parent, F_1, and F_2 populations planted on February 1, 1945, are summarized in table 3. All parent and F_1 plants flowered but only

TABLE 3

FREQUENCY DISTRIBUTION WITH RESPECT TO NODE NUMBER AT WHICH FIRST FLOWERING OCCURRED FOR TWO VARIETIES OF CASTOR BEAN AND THEIR F_1 AND F_2 PROGENIES. EXPERIMENT IV*A*. PLANTED IN GREENHOUSE FEBRUARY 1, 1945. FINAL DATA COLLECTED SEPTEMBER 17, 1945

Location of first flowers (node no.)	Character of population									
	Parent		F_1	F_2						
	Ky. 38	D.P. 227	X_3	$X_{3\text{-}5}$	$X_{3\text{-}6}$	$X_{3\text{-}7}$	$X_{4\text{-}3}$	$X_{4\text{-}4}$	$X_{4\text{-}5}$	Total
6	.....	6	.....	1	.....	.....	.....	.....	.....	1
7	.....	40	1	27	6	7	4	2	8	54
8	.....	14	4	42	10	15	16	4	17	104
9	.....	.....	6	17	1	7	9	5	5	44
10	.....	.....	1	21	2	5	10	13	4	55
11	7	.....	1	24	5	6	23	2	11	71
12	6	.....	4	9	3	3	8	1	7	31
13	12	.....	2	6	3	2	6	1	3	21
14	6	.....	.....	4	.....	2	3	2	1	12
15	2	.....	.....	5	1	4	3	2	2	17
16	1	.....	.....	4	.....	1	3	4	1	13
17	.....	.....	.....	2	1	1	2	2	3	11
18	.....	.....	.....	5	1	1	1	.....	.....	8
19	.....	.....	.....	2	.....	1	3	1	.....	7
20	.....	.....	.....	4	.....	2	2	1	1	10
21	.....	.....	.....	3	.....	1	3	1	1	9
22	.....	.....	.....	1	.....	.....	2	.....	.....	3
23	.....	.....	.....	.....	.....	.....	1	.....	.....	1
24	.....	.....	.....	1	.....	.....	.....	.....	1	2
25	.....	.....	.....	2	1	.....	.....	.....	.....	3
26	.....	.....	.....	3	.....	.....	.....	.....	.....	3
27	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	.....	.....	.....	1	.....	.....	.....	.....	.....	1
29	.....	.....	.....	1	.....	.....	1	.....	.....	2
30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
31	.....	.....	.....	1	.....	.....	.....	.....	1	2
32	.....	.....	.....	.....	.....	.....	.....	.....	1	1
33	.....	.....	.....	1	.....	.....	.....	.....	.....	1
34	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
35	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
36	.....	.....	.....	.....	.....	1	.....	1	.....	2
37	.....	.....	.....	.....	.....	1	.....	.....	.....	1
38	.....	.....	.....	1	.....	.....	.....	.....	.....	1
Flowerless	0	0	0	21	0	12	14	3	5	55
Total	34	60	19	209	34	72	114	45	72	546
% flowerless	.....	.....	.....	10.0	0.0	16.7	12.3	6.7	6.9	10.1
Av. fl. node	12.8± 0.2*	7.1± 0.1*	9.9± 0.4	11.6	10.5	11.7	12.1	12.4	11.3	11.7

* For parent populations, significantly different from each other and each from F_1 at the 1% level.

89.9% of the F_2 plants. The percentage of flowerless F_2 plants in this study is similar to the 8% obtained in the field (5) on F_2 material from the same parents.

The average node positions of first flowers for the parents, Kentucky 38 and D.P. 227, were significantly different from each other and from the average position in the F_1 population, which was intermediate between the two parents.

In only one of the six F_2 populations, namely, X_3-6, which was the smallest one, did all plants flower or form primordia. Among the remaining five lines, the percentages of flowerless plants ranged from 6.7 to 16.7. Disregarding the flowerless plants, none of the F_2 populations differed from one another with respect to the average node position of the first flower primordia. The greatest number of nodes per plant on the flowerless individuals dissected in September was 45; the least, 33.

On similar material planted in the field during 1944, DOMINGO (5) observed an average of 8, 10, 10, and 10 nodes for the position of first flower primordia on plants of Kentucky 38, D.P. 227, F_1, and F_2, respectively. The present data for F_1 and F_2 approximate his results, but the results for the parents were outstandingly different. In the earlier data (5) the parental means differed by two nodes, D.P. 227 having the greater mean. In the present study the mean for Kentucky 38 was five nodes greater than that for DP. 227. A possible explanation for these differences in results is considered in reporting subsequent experiments.

EXPERIMENT *IVB*.—At approximately monthly intervals following the planting of February 1, 1945, four additional plantings of both Kentucky 38 and D.P. 227 parent seed were made in 8-inch pots in the greenhouse. These plants were likewise grown on $14\frac{1}{2}$-hour photoperiods until May 6, when the natural daylength exceeded $14\frac{1}{2}$ hours, and all plants were put on natural photoperiod alone. The last two monthly plantings were grown only with natural photoperiod, since the natural daylength was already in excess of $14\frac{1}{2}$ hours.

The average node position of first flowers on Kentucky 38 in the February 1 planting was 12.8 (table 4), which was four nodes higher than in experiment I. With each successive planting of this variety, the first flowers were formed at either the same or at a lower mean position. The mean positions of first flowers for each of the first two plantings were significantly higher than those of each of the last two plantings. These differences may have been correlated with the fact that the last two plantings had received solely natural daylight which exceeded $14\frac{1}{2}$ hours after May 6, while the earlier lots received a fixed $14\frac{1}{2}$-hour photoperiod, a portion of which was Mazda light.

The results for D.P. 227 for the five planting dates were in contrast to those obtained with Kentucky 38 in both the degree and the direction of change of the node position of first flowers. With D.P. 227 there was a substantial increase in this mean node value with the successive planting dates. These increases were all statistically significant at the 1% level of probability. In the case of the parents planted on February 1, 1945, the average node position of first flowers for Kentucky 38 plants was higher than for D.P. 227 plants (table 4). This relationship was the reverse of what DOMINGO (5) reported for these varieties grown in the field during the summer. However, in the case of the fifth planting made on May 26, 1945, in the greenhouse, the relative location of the first flower node in the two varieties was the reverse of

that obtained with the February lots and was then like that reported for the field-grown material (5), with first flowers of D.P. 227 plants at a higher node position than were those of Kentucky 38. This differential trend in node values for these varieties took place simultaneously with changes in duration of natural daylight occurring through the growing period.

whether flowerless F_2 plants could be made to flower if grafted to those of other types which flower readily. It is known in the case of soybeans that plants of the Biloxi variety, which are vegetative with long photoperiods, flower readily on such photoperiods if grafted to those of the Agate variety which normally flower on long daylengths (7).

TABLE 4

FREQUENCY DISTRIBUTION WITH RESPECT TO NODE OF FIRST FLOWERING FOR TWO VARIETIES OF CASTOR BEAN PLANTED ON FIVE DIFFERENT DATES. PLANTS GROWN IN GREENHOUSE ON 14½-HOUR PHOTOPERIOD UNTIL MAY 1, 1945, AND SUBSEQUENTLY ON NATURAL PHOTOPERIOD. EXPERIMENT IV*B*

Planting date	Node at which first flower primordia developed											Total plants	Average
	6	7	8	9	10	11	12	13	14	15	16		
	Kentucky 38												
2- 1-45						7	6	12	6	2	1	34	12.8±0.2*
3- 3-45				5	5	2	6	8	21	7	1	55	12.9±0.2*
4- 2-45				2	5	7	10	12	10	3		49	12.4±0.2
5- 1-45					6	10	14	9	3			42	11.8±0.2
5-26-45				2	9	7	8	7	2		4	39	11.9±0.3
	D.P. 227												
2- 1-45	6	40	14									60	7.1±0.1†
3- 3-45		7	25	10	2							44	8.2±0.1†
4- 2-45		1	14	28	11		2					56	9.0±0.1†
5- 1-45			2	16	21	8	1					48	9.8±0.1†
5-26-45							11	12	9	3		35	13.1±0.2†

* Significantly different at the 1% level from 5–1–45 and 5–26–45 plantings of Kentucky 38.
† Significantly different at the 1% level from all other plantings of D.P. 227.

EXPERIMENT IV*C*.—A number of reciprocal grafts were made involving apparently flowerless F_2 individuals planted on February 1, 1945, and plants of the Kentucky 38 parent. The F_2 plants were grown in a soil bed in the greenhouse on 18-hour photoperiod. The Kentucky 38 plants were planted in pots on May 1, 1945, and had been growing vegetatively with an 8-hour photoperiod. The purpose of the grafts was to determine

The first bud grafts were made on June 30, 1945. In all such grafts lateral buds were employed. With both stock-scion combinations the terminal bud and all but one lateral bud above the location of the graft were removed from the stock. Most of these grafts were successful, and satisfactory growth of the scion occurred in all such cases (fig. 2*A*). In one instance an approach graft was made, a flowerless F_2 plant serving final-

ly as the stock and a Kentucky 38 plant as the scion (fig. 2*B*).

In all cases the Kentucky 38 plant flowered, and the flowerless F_2 plant remained vegetative, regardless of the variety used as stock or scion. In all cases the first flower primordia to appear on Kentucky 38, whether serving as stock or scion, were developed at a substantially higher node number than could be accounted for by the number of nodes present in the initial bud.

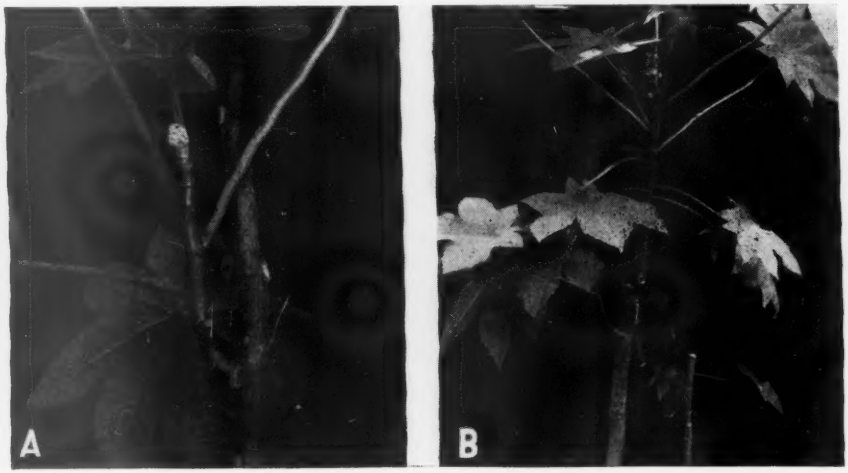

FIG. 2.—Grafts between Kentucky 38 variety parent and a flowerless F_2 plant, the former serving as scion and the latter as stock. *A*, bud graft with F_2 stock vegetative and the scion showing young inflorescence. *B*, approach graft with F_2 stock vegetative and the scion showing elongated inflorescence. (Photographed September 14, 1945.)

EXPERIMENT V

Plants of Kentucky 38 did not flower when grown in the greenhouse during winter periods in which the natural daylength was shortest and frequently of lowest average light intensity, nor did they flower during these periods when incandescent light of 25 foot-candles intensity supplemented the natural daylength to provide 24-hour photoperiods. However, during the months of March through August all the Kentucky 38 plants flowered, and the first flowers were formed at a lower average node position on those that were grown during the middle of this period when the longest natural daylengths occurred. Such data suggest that flowering of this species may be influenced both by length of photoperiod and by total radiation received during the photoperiod. The influence of these two factors was therefore investigated further.

EXPERIMENT V*A*.—In this experiment the factors of duration and intensity of light were investigated for Kentucky 38 and D.P. 227 at a time of year in which both varieties had previously flowered. On July 21, 1945, seed was planted outdoors in four soil beds with twenty plants of each variety in each bed. Two beds were subjected to full natural daylength, the other two beds to 9 hours of natural daylight. Differential photoperiods were begun immediately with germination of seed and were

maintained until September 5, 1945, at which time node counts were made (table 5). All plants flowered when subjected to 9-hour and to natural photoperiods; but the Kentucky 38 plants flowered at a significantly higher node position with the 9-hour photoperiod than with natural photoperiod, whereas the reverse relationship was found among the D.P. 227 plants.

EXPERIMENT V*B*.—The effect of several different light intensities upon the earliness of flowering of Kentucky 38 variety was measured by the node number at which first flowers developed. On August 18, 1945, seed of Kentucky 38 was planted outdoors in six soil beds following the procedure used in experiment V*A*. Light intensities of 100, 70, and 30% of that available naturally were applied in duplicate, the lower intensities being obtained by shading with different numbers of layers of tobacco cloth.

The plants were grown until October 3, 1945, at which time either the node position of first flowers or the total number of nodes on nonflowering plants was recorded for each plant. Duplicate fresh-weight and quadruplicate dry-weight data were obtained on tops cut at the cotyledonary node (table 6).

Obviously significant differences in the flowering responses resulted with the different treatments. No plants flowered when grown with 30% of full light intensity, although they had produced an average of 14.2 nodes per plant. With 70% intensity, fifty-two of the sixty-two plants flowered with first flowers at the average node position of 10.4. The nonflowering plants developed an average of 15.5 nodes in their main stems. All plants flowered when grown with 100% of the natural light available, and the average node at which first flowers occurred was 9.0. The difference in the average position of first flowers was statistically significant. The same was true for total nodes on the nonflowering plants receiving the two lowest intensity treatments.

Statistically significant differences in average fresh-weight and in dry-weight percentage were found among plants that received different intensities. The highest average fresh weight occurred with the 70% intensity, the lowest with the 30% intensity. The presence of the highest average fresh weight in plants of the 70% lot may possibly be accounted for by the differences in flowering response between this lot and the 100% one. The delayed flowering response obtained with

TABLE 5

EFFECT OF PHOTOPERIOD UPON INITIATION OF FLOWER PRIMORDIA IN KENTUCKY 38 AND D.P. 227 VARIETIES OF CASTOR BEAN GROWN IN OUTDOOR SOIL BEDS. EXPERIMENT V*A*. PLANTED JULY 21, 1945. HARVESTED SEPTEMBER 5, 1945. FORTY PLANTS PER LOT

VARIETY	AVERAGE NODE AT WHICH FIRST FLOWER PRIMORDIA OCCURRED	
	Natural daylength	9-hour photoperiod
Kentucky 38	8.7±0.1*	12.0±0.1
D.P. 227	9.7±0.1	8.9±0.1*

* These two lots are not significantly different from each other. All other differences significant at the 1% level of probability.

plants of the 70% lot in contrast to the 100% lot, plus the fact that a number of the former were still vegetative at harvest, could account for its greater fresh weight.

Discussion

Relatively little has been reported concerning the effects of either duration of photoperiod or intensity of light upon the flowering of castor bean. ADAMS (1, 2) observed the effect of these two factors upon the flowering of several species of plants including the castor bean but did not name the variety of castor bean employed. In one of his experiments with light intensity all plants flowered when grown solely with continuous light from 300-watt or 500-watt nitrogen-filled lamps. Investigation of the effects of duration of light (2) was confined to extremely short photoperiods, not exceeding 3 hours of natural light daily. The results of his study were not conclusive, since approximately half of the plants of each photoperiod lot failed to flower at the end of a 3-month growing period.

The present studies indicate that both duration of photoperiod and light intensity are factors which may influence formation of flower primordia in certain varieties of castor bean. This species thus seems to be different from Biloxi soybean (4) and *Xanthium pensylvanicum* (6), which are little influenced in flowering by rather wide differences in total radiant energy but are very sharply influenced by small differences in daylength. These plants remain vegetative if grown on photoperiods that exceed a certain critical length and flower promptly if grown on shorter ones. The intensity of supplemental light used to extend the natural photoperiod, and so prevent flowering of such plants, need not be more than 25 foot-candles and can be as low as a fraction of 1 foot-candle.

With plants of Kentucky 38 variety, formation of flower primordia is apparently dependent upon a total light-energy factor, and thereby both duration and intensity of light are involved. Measured in terms of node number at which first flowers were formed, the earliest flowering occurred when plants were grown on photoperiods of natural length or longer, during the spring, summer, and fall months, a period of the year having longest natural daylengths and relatively high average daily light intensity. Plants grown during these same periods on 8-

TABLE 6

EFFECT OF LIGHT INTENSITY UPON PLANTS OF KENTUCKY 38 VARIETY CASTOR BEAN GROWN IN OUTDOOR SOIL BEDS ON NATURAL PHOTOPERIOD. EXPERIMENT V*B*
PLANTED AUGUST 18, 1945. HARVESTED OCTOBER 3, 1945

LIGHT INTENSITY (%)	NO. OF PLANTS	AV. FRESH WEIGHT (GM.)	DRY WEIGHT* (%)	FLOWERING PLANTS		VEGETATIVE PLANTS	
				No.	Av. first flower node	No.	Av. total no. of nodes
100	57	76.8±2.3†	10.57±0.5	57	9.0±0.02‡	0	.....
70	62	97.8±0.8	10.03±0.4	52	10.4±0.02	10	15.5±0.2‡
30	52	34.1±0.9§	9.48±0.5	0	.....	52	14.2±0.1

* Figures are averages, each derived from four 200-gm. fresh-weight samples.
† Significantly different from 70% intensity lot at the 5% level.
‡ For average first flower node and average number of nodes, significantly different from other treatment at the 1% level.
§ Significantly different from other two lots at the 1% level.

hour natural photoperiod remained vegetative. Likewise plants grown during the winter months of shortest natural daylengths and low average daily light intensity failed to form flower primordia with any duration of daily photoperiod or even with continuous light. During this period of the year extension of the natural photoperiod with incandescent-filament light of 150 foot-candles was not effective in promoting floral initiation. However, when plants were grown for 7 weeks during winter months with 15-hour or longer photoperiods in a controlled-environment room in which the light intensity was about 2000 foot-candles, flower primordia were formed on ten of a total of twenty-eight individuals.

These data indicate that a relatively high level of light energy is a major requirement for floral initiation in plants of Kentucky 38 variety. It was not possible to determine the quantity of light energy required, but, in terms of duration of photoperiod and intensity of light, several of the combination requirements have been established for definite seasons of the year. D.P. 227 differed from Kentucky 38 variety in that it flowered regardless of duration of photoperiod on which it was grown. With photoperiods conducive to flowering, first flowers in Kentucky 38 occurred at successively lower node positions as the daylength increased, while the reverse trend was true for first flowers of D.P. 227 plants grown with these daylengths.

All attempts to induce formation of flower primordia on flowerless F_2 plants resulting from crosses of Kentucky 38 and D.P. 227 parents were unsuccessful. On the basis of the flowering responses of the parents to different photoperiods and light intensities, as well as the prompt flowering of F_1 individuals, it does not appear probable that the flowerless F_2 plants lack the capacity to form flowers. More likely other factors of the environment, such as temperature and nutrition, may be involved in controlling formation of flower primordia, along with the light-energy factor effective in the case of the Kentucky 38 and D.P. 227 parents. Significantly higher intensities of light occur in tropical regions, to which castor bean is indigenous, than in temperate regions. ALLEE (3) reported maximum light intensities of the order of 20,000 foot-candles in a series of observations over several weeks at Barro Colorado Island in Panama as compared with 14,000 at Chicago, Illinois; but a great many variables are involved, and these figures admittedly are not a basis for exact comparison. It may be possible that the flowerless F_2 plants require even higher light intensities than do Kentucky 38 plants—intensities such as prevail in tropical regions.

Summary

1. Studies were made of the effects of photoperiod and light intensity upon flower formation in plants of several genetically different populations of castor bean. Plants were grown in soil in the greenhouse, field, or controlled-environment rooms. The node position of the first flowers and the total number of nodes on nonflowering plants were the criteria used for measuring the responses of plants to duration and intensity of light.

2. In experiments concerned with photoperiod alone, marked variation in degree of flowering response was observed for a number of varieties. Some failed to flower on either long or short photoperiods, while those of other varieties flowered on both photoperiods. In

the case of one variety, flowers developed only on plants grown with long photoperiod.

3. Plants of two parents and their F_1 and F_2 progenies, the last of which contained plants that remain flowerless when grown under normal field conditions at Beltsville, Maryland, were grown with controlled photoperiod. Plants of both parents and the F_1 progeny flowered at relatively low node positions, the F_1 plants being intermediate between the parents in this character. The node position of first flowers on most of the F_2 plants was within the range of that of the parents and the F_1 progeny, but 10% of the F_2 plants remained vegetative under the several treatments given.

4. Flowerless F_2 plants remained vegetative when grown with several different photoperiods and likewise when grafted either as stock or as scion with vegetative parent plants which subsequently were induced to flower.

5. Earliness of flowering of parent plants whose F_2 populations contain the flowerless character was significantly affected by variation in photoperiod or in light intensity. In the case of one parent, flowering was markedly controlled by a light-energy factor. In this case floral initiation occurred only on long photoperiods applied during spring, summer, and fall. During the short days of winter, flowers failed to form even under a continuous 24-hour photoperiod in which supplementary artificial illumination was supplied.

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INFLUENCE OF POTASSIUM AND SODIUM ON METABOLISM OF PEANUT COTYLEDONS DURING GERMINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **585**

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Introduction

It had been noticed that, when cocklebur was grown under potassium-deficient conditions, the cotyledons persisted in a fleshy and turgid condition much longer than did those of control plants. It was conjectured that this persistence might be a result of impedance of the vascular transport of food reserves from the cotyledons, since both breakdown (**15**) and interference with formation of phloem tissue (**26**) have been described as potassium-deficiency symptoms.

An experiment was designed to investigate the relative rates of removal of the important cotyledonary food reserves

under conditions of adequate (+K) versus no (−K) potassium supply, as well as to study other symptoms associated in the −K syndrome. Since seeds of cocklebur were unavailable in large quantities, Virginia peanut seed was chosen as the test object. It has the advantages of being large, fatty, and epigeal in germinating habit. The experiments were carried out in the dark to avoid confusion of the interpretation by the addition of new products of photosynthesis.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS USED

Salts (Stock solutions)	Solution A (+K−Na)	Solution B (−K+Na)	Solution C (−K−Na)
$Ca(NO_3)_2$ 0.05M	20 ml./l.	20 ml./l.	20 ml./l.
$MgSO_4$ 0.05M	10 ml./l.	10 ml./l.	10 ml./l.
KH_2PO_4 0.05M	10 ml./l.	.....	.....
NaH_2PO_4 0.05M	.....	10 ml./l.	.....
$Ca(H_2PO_4)_2$	.....	.....	(0.0025M)
H_3BO_3 0.0463M $MnCl_4$ 0.0064M $ZnSO_4$ 0.00076M $CuSO_4$ 0.0003M H_2MoO_4 0.0004M	1 ml./l.	1 ml./l.	1 ml./l.
$Fe_3(C_4H_4O_6)_3$ 0.0089M	1 ml./l.	1 ml./l.	1 ml./l.
Atmospheres	1.12	1.12	1.05

Material and methods

In each experiment Virginia peanuts obtained from a commercial seedsman were shelled, examined, and selected for undamaged seeds. They were planted in washed quartz sand in glazed pots, the drains of which were covered with glass wool. Each pot was watered before and after planting and thereafter daily or every second day, depending on the experiment, with its appropriate nutrient solution. Nutrient solutions (table 1) were based on those suggested by HOAGLAND and ARNON (9). The pots were kept in a basement room of relatively even temperature, and the room was maintained in complete darkness except at times of watering and harvest. These operations were carried out by the light of a 15-watt blue bulb shielded except at the tip and carried in the hand. The plants were grown for 6 (experiment I) or 7 (experiments II and III) and for 15 days (all experiments).

At harvest the sand was washed from the root systems. It was found impossible, however, either to retain all the brittle lateral roots of the 15-day plants or to remove the sand from the roots completely by washing and rinsing with concentrated sodium chloride solution. Therefore, the root systems of the 15-day plants were severed from the hypocotyls at the point of divergence of the first lateral root and were discarded.

Immediately at harvest the wet weights of the 6- or 7-day cotyledons and axes (hypocotyl plus epicotyl) and of the 15-day cotyledons, hypocotyls, and epicotyls were taken separately. The axial tissues were dried in an air-blast oven at 150° C. for 5 minutes; then the temperature was reduced to 70° C. to complete the drying. After drying for 4 hours, they were placed in desiccators and held until weighed. The fresh cotyledons from each treatment were harvested in triplicate lots, weighed in pairs, and the lot members mixed together. Each lot was ground

in a meat-chopper with 2 gm. of calcium carbonate for each 100 gm. of tissue in such a manner that the discharge from the mill dropped into boiling redistilled ethanol in quart mason jars. The jars were sealed and held in a dark cabinet until the contents could be analyzed.

Methods for chemical analyses were those described by LOOMIS and SHULL (13), except that lipids were determined by the method of SANDO (20) and lipolytic activity was estimated by the method of LONGENECKER and HALEY (12) on fresh cotyledons. Invert sugars and crude fiber were both determined by difference (total sugar − reducing sugars = invert sugars; total dry matter − dry weight of determined substances = crude fiber). Comparisons of wet-weight and dry-weight data were made by the analysis of variance technique described by SNEDECOR (23).

Material for histological study was killed in CROOKS's modification of Navashin's solution (3), washed in tap water for 48 hours, dehydrated in a tertiary butanol–ethanol series, imbedded in paraffin, sectioned at 15 μ, and stained with Flemming's triple stain.

DESCRIPTION OF EXPERIMENTS

In experiment I, selected peanut seeds were further selected by rejecting all seeds falling outside the limits of ± 0.05 gm. of the mean seed weight. The seeds were planted as described above, and either solution A or solution B (table 1) was applied daily. Three lots of each treatment were harvested at 6 days after planting and three more lots at 15 days. Analyses were made for lipids on the absolute basis only.

Experiment II was performed similarly to experiment I, except that the first harvest was taken on the 7th rather than on the 6th day after planting. Analyses were made of these samples of cotyledons for moisture, total sugars, reducing sugars, starch and dextrins, acid-hydrolyzable substances, soluble nitrogen, insoluble nitrogen, and total lipids.

Experiment III was designed similarly to experiments I and II, with the following exceptions: three instead of two nutrient solutions were used: A, B, and C (table 1); pots were treated with nutrient solution every second day; and the seeds, instead of being merely selected for size, as in experiments I and II, were, in addition, individually weighed and their weights recorded. Solution C was designed to furnish the same amount of $H_2PO_4^-$ ions as solutions A and B, to supply neither potassium nor sodium, to cause as small a difference as possible from the other two solutions in the concentration of calcium, and to have approximately the same ionic concentration as the other solutions. Plants were again harvested at 7 and at 15 days after planting. In this experiment wet weights of the 7-day cotyledons and axes and of the 15-day cotyledons, hypocotyls, and epicotyls were taken; dry weights of 7-day axes and of 15-day hypocotyls and epicotyls were also recorded. Dry weights of cotyledons could not be obtained because of the analytical procedure used.

Results

Removal of the two predominant complex reserve materials, fats and proteins, from the cotyledons proceeded somewhat slowly for the first 6 or 7 days after planting but went on much more rapidly during the next 8 days (fig. 1). It is assumed from the data (tables 2–4) and from the fact that cotyledons of etiolated seedlings were not visibly necrotic until 4 weeks after planting that the quantity of lipid and protein reserves remaining in peanut cotyledons during germination

and early growth follows an inverse sigmoid curve with time the abscissa. The effect of substitution of sodium for potassium in the nutrient substrate appears to be to flatten this curve; this is most marked at the 7-day point. Wider differences are evident between treatments in dry weight of cotyledons, dry weight of entire plants, and grams of lipid and protein per 100 plants at the mid-point of the experimental period than at the end. The 7-day plants had less dry matter both in axes and in cotyledons, and less lipid and protein in the cotyledons under −K +Na conditions (solution B) than under +K −Na (solution A). Although carbohydrates in the cotyledons, especially starch, increased somewhat during germination, the increase was not in the same ratio in experiments II and III, nor was it consistent with treatment. There was a reversal between the 7th and 15th day in the distribution of lipids between treatments in all three experiments: whereas more lipid remained in the +K −Na cotyledons at 7 days, by the

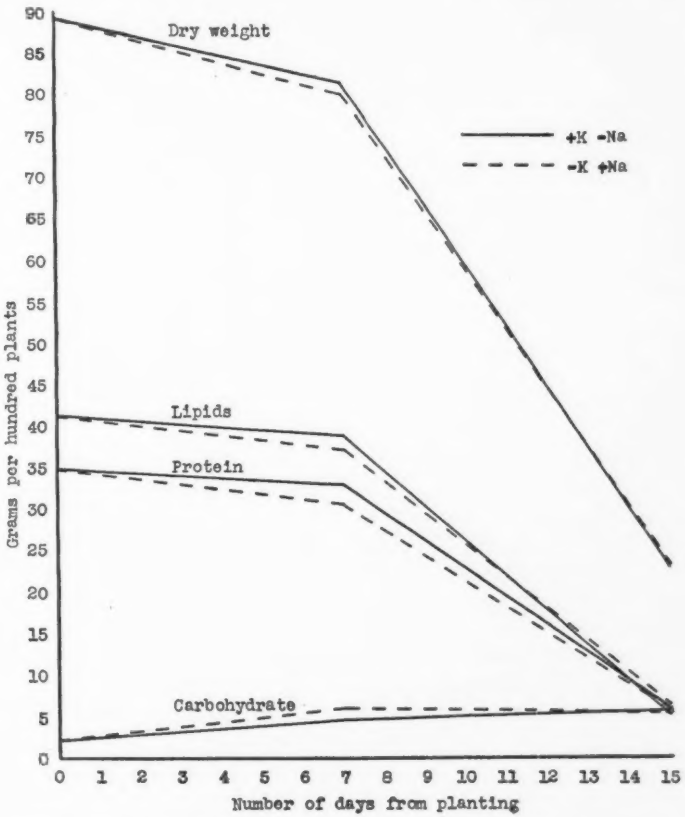

FIG. 1.—Changes in amount of dry weight, carbohydrates, lipids, and proteins in cotyledons of Virginia peanuts during first 15 days after planting in dark as affected by substitution of sodium for potassium (data of experiment II).

15th day they had lost more lipid than the −K +Na cotyledons. When both potassium and sodium were eliminated from the nutrient solution (solution C, experiment III), lipids were not so rapidly lost as when either was present, and the amounts of starch found in the cotyledons more nearly approximated those for the +K −Na treatment.

Statistical analysis of the wet- and dry-weight data for 15-day plants from experiment III (table 4) revealed highly significant differences owing to treatment between wet epicotyl weights, between wet weights of entire plants (without roots), and between dry epicotyl weights. Differences significant at the 5% point were found between wet weights of axes (without cotyledons and roots), between dry weights of axes, and between wet weights of cotyledons. Differences between treatments of original seed weights were not significant, nor were differences between dry weights of 15-day hypocotyls, nor between any wet or dry weights of parts or complete plants at 7 days.

There was no visible difference among the roots of plants from the various treatments. Histological study of the vascular tissue of the cotyledonary petiole revealed no evidence of breakdown and no differences between treatments in amount of phloem present. Tests of lipolytic activity performed *in vitro* on fresh cotyledons using peanut oil as a substrate showed no differences in fat-hydrolyzing rate between treatments.

TABLE 2

ANALYTICAL DATA, EXPERIMENTS I AND III, GRAMS PER 100 PLANTS

	Experiment I	Experiment III			
	Total lipids	Dry weight	Moisture	Starch and dextrins	Total lipids
Seeds	36.31	89.01	18.52	0.92	41.26
6- or 7-day cotyledons:					
+K −Na	18.87	76.37	68.27	4.72	26.96
−K +Na	17.78	70.72	77.06	3.59	22.26
−K −Na		74.77	73.83	4.63	27.58
15-day cotyledons:					
+K −Na	3.83	35.90	55.37	2.70	6.18
−K +Na	4.62	37.05	58.63	2.42	6.33
−K −Na		36.79	60.34	2.93	7.33

Discussion

When potassium was lacking from the nutrient medium during germination of Virginia peanuts, certain well-known potassium-deficiency symptoms became recognizable as early as the 7th day after planting. It is especially interesting that these symptoms were induced in such a short time in a seedling which, according to analyses cited by WINTON and WINTON (**27**), contains 35–40% K_2O in the ash of the seed.

In the following discussion of the condition and metabolism of the cotyledons the writer has placed more emphasis on the middle part of the period covered by the experiments (although not to the exclusion of the later conditions); it is thought that their metabolism during

TABLE 3

ANALYTICAL DATA, EXPERIMENT II

	Dry weight	Moisture	Reducing substances	Invert sugars*	Starch and dextrins	Acid-hydrolyzables	Soluble nitrogen	Insoluble nitrogen	Total protein	Total lipid	Total carbohydrate	Crude fiber†
Seed:												
% of dry weight		17.22‡	0.02	1.15	1.10	None	0.06	6.28	39.26	48.95	2.27	9.46
Gr./100 plants	89.01	18.52	.02	0.97	0.92	None	0.05	5.59	34.94	41.26	1.91	8.42
7-day cotyledons (+K−Na):												
% of dry weight		50.16‡	.08	1.70	4.01	None	0.05	6.44	40.23	47.19	5.79	6.74
Gr./100 plants	81.79	82.33	.06	1.40	3.28	None	0.04	5.26	32.90	38.60	4.74	5.51
7-day cotyledons (−K+Na):												
% of dry weight		51.23‡	.10	1.82	5.38	None	0.05	6.17	38.55	46.41	7.30	7.69
Gr./100 plants	79.98	83.99	.09	1.53	4.30	None	0.04	4.93	30.83	37.12	5.92	6.15
15-day cotyledons (+K−Na):												
% of dry weight		78.33‡	.40	4.29	19.65	0.39	1.12	4.02	25.10	22.06	24.73	26.99
Gr./100 plants	22.82	82.50	.09	0.98	4.48	0.09	0.26	0.92	5.73	5.03	5.64	6.16
15-day cotyledons (−K+Na):												
% of dry weight		76.50‡	.23	3.67	18.02	0.33	0.96	4.02	24.79	27.16	22.25	24.84
Gr./100 plants	23.10	75.20	0.05	0.85	4.16	0.07	0.22	0.93	5.81	6.27	5.13	5.74

* Determined by difference: total sugars − reducing sugars = invert sugars.

† Determined by difference: total dry matter − total dry weight of determined substances = crude fiber.

‡ Per cent of total weight.

TABLE 4

WET AND DRY WEIGHT DATA, EXPERIMENT III

Material	Treatment	Mean weight (gm.)	Standard deviation	F	Degrees of freedom
*7-day plants:**					
Original seeds, air-dry	+K−Na	0.869	0.072	1.315	2, 105
	−K+Na	0.860	.067		
	−K−Na	0.889	.086		
Cotyledon pairs, wet	+K−Na	1.431	.133	1.376	2, 105
	−K+Na	1.444	.128		
	−K−Na	1.469	.137		
Axes plus cotyledons, wet	+K−Na	3.006	.279	1.151	2, 105
	−K+Na	2.928	.260		
	−K−Na	2.986	.230		
Axes alone, wet	+K−Na	1.551	.251	1.076	2, 105
	−K+Na	1.472	.220		
	−K−Na	1.509	.211		
Axes alone, air-dry	+K−Na	0.160	.098	2.329	2, 105
	−K+Na	0.146	.024		
	−K−Na	0.152	.026		
15-day plants:†					
Original seeds, air-dry	+K−Na	0.858	.092	2.105	2, 97
	−K+Na	0.898	.104		
	−K−Na	0.858	.075		
Cotyledon pairs, wet	+K−Na	0.849	.183	4.509‡	2, 97
	−K+Na	0.941	.216		
	−K−Na	0.991	.164		
Axes (without roots) plus cotyledons, wet	+K−Na	5.615	.796	7.066§	2, 97
	−K+Na	5.292	.772		
	−K−Na	4.906	.578		
Axes without roots or cotyledons, wet	+K−Na	4.721	.671	4.760‡	2, 97
	−K+Na	4.374	.853		
	−K−Na	4.067	.929		
Axes without roots or cotyledons, air-dry	+K−Na	0.422	.066	4.692‡	2, 73
	−K+Na	0.401	.052		
	−K−Na	0.369	.067		
Epicotyls, wet	+K−Na	2.402	.428	10.899§	2, 97
	−K+Na	2.139	.388		
	−K−Na	1.872	.370		
Epicotyls, air-dry	+K−Na	0.229	.046	6.325§	2, 93
	−K+Na	0.215	.034		
	−K−Na	0.195	.032		
Hypocotyls, without roots, air-dry	+K−Na	0.186	.039	1.933	2, 77
	−K+Na	0.187	.034		
	−K−Na	0.176	0.044		

* Based on thirty-six plants in each treatment.

† Based on twenty-three to thirty-five plants in each treatment.

‡ Significant at odds of 19:1. § Significant at odds of 99:1.

this earlier period was more free than during the later period from the effects of the hunger metabolism which supervenes as the cotyledonary reserves become exhausted and which might be expected to mask the effects of the treatments (22).

DRY MATTER.—Dry weights of cotyledons and plant axes at 7 days were less under −K −Na and much less under −K +Na conditions than in the controls (tables 2–4). This is interpreted as confirming the frequently reported symptom of potassium deficiency—exalted respiratory rate (7, 19, 25). In addition, it indicates that the presence of large amounts of available sodium in the absence of much potassium induces a higher respiratory rate than is shown by plants deprived of both sodium and potassium. Such a situation has not come to the writer's attention through the literature. This differential in loss of dry weight between treatments is paralleled by a differential in rates of utilization of lipid and protein reserves.

Dry weights of cotyledons at 15 days were least under +K −Na and greatest under −K +Na conditions; this apparently is analogous to the situation earlier observed in cocklebur. At the same time, weights of plant axes and entire plants were greatest under +K −Na and least under −K −Na conditions. It is thought that this change in relationships compared with the status at 7 days was due to the change in character of growth between the 7th and 15th days; during this period the entire lateral root system of the seedling was developed. At the 7th day the primary root was short, smooth, and branchless, while at the 15th day its upper half was covered with laterals varying in length from 3 to 4 cm. at the base of the hypocotyl to mere swellings at the mid-length of the primary root. The development of so many additional terminal meristems would impose a seriously accelerated demand on the potassium reserves of the seed (5, 15, 21); it could thus bring on not only the reversal of relative respiratory rates between the two treatments but the stunting of the epicotyls of the −K −Na plants in comparison with the other two (+K −Na and −K +Na) treatments. In the −K +Na plants the situation is not so severe, probably because of the effectiveness of sodium in partially relieving the potassium deficiency (1, 8, 11).

Moisture contents of cotyledons (tables 2, 3), of axes, and of entire plants at 7 days were slightly higher in −K +Na than in other treatments. Differences in moisture content between treatments at 15 days were not consistent in experiments II and III. In general, the moisture content differences were small and afforded little basis for conclusions with respect to the effect of potassium deficiency on turgidity of tissues.

CARBOHYDRATES.—Carbohydrates are of minor importance from a quantitative standpoint among the peanut seed reserves, as they constituted only 2.27% of the dry weight of the cotyledons. Data for carbohydrates in the cotyledons also show conflict between experiments II and III; this may have been because of slight differences in experimental conditions between experiments. Such differences would be accompanied by small differences in metabolic states of the developing tissues which might have been great enough to cause the conflicting results.

In experiment II the amount of carbohydrate in the cotyledons was inversely related to the amount of lipid remaining; this is what would usually be expected from a consideration of the results of PETERS (16), LASKOVSKI (10), MILLER

(14), and others with germinating fatty seeds. This was not confirmed, however, by experiment III.

In contrast to the observation of DAY (4), but in agreement with that of NIGHTINGALE *et al.* (15) on tomato stems, there was no apparent interference with the plant's ability to form starch in these experiments under −K +Na nutrient conditions, since appreciably more starch was found in the cotyledons at 7 days (experiment II) and in the histological sections of −K +Na petioles and hypocotyls at both 7 and 15 days than in the +K −Na plants.

PROTEIN.—The fate of the protein reserves was similar to that of the other complex reserves. The cotyledons behaved like the senescent leaves described by RICHARDS and TEMPLEMAN (19) in that proteolysis proceeded rapidly, thus making the amino acids available to the growing axis. The rate of protein loss from the cotyledons was increased by −K +Na conditions compared with the control (fig. 1, table 3) during the earlier period of growth; eventually the values for protein remaining in the cotyledons under the two treatments drew together again as the reserve protein approached exhaustion at the 15th day. This situation is similar to those described by RICHARDS (17) and by WALL (25), in which high respiratory rate and −K +Na nutrition were combined; in this case, however, they were not accompanied by more soluble nitrogen, at least in the cotyledons, probably because all available soluble nitrogen compounds were being used in protein synthesis in the rapidly growing axis. This would be expected, especially if potassium is necessary specifically for the early stages of nitrate reduction (15) or for the conversion of ammonium to other soluble nitrogen compounds (18), but not if potassium is necessary only for the final stages of protein synthesis from amino acids (7, 25). In this regard the writer follows the opinion expressed by TIEDJENS and WALL (24) in discussing the effect of potassium deficiency on carbohydrate formation—that these effects are "due to injury to the protoplasm because ion balance is lacking rather than to any specific unfilled catalytic role of potassium." In other words, the imbalance resulting from the substitution of potassium by sodium or any other ion disturbs protoplasmic function along a wide front, which seems plainly evident from the results of these experiments.

LIPIDS.—The curve representing the rate of loss of lipids from the cotyledons (fig. 1) parallels that for the decrease in amount of protein present, except that the rate was slightly faster over the 15-day period. In the case of lipids, as with proteins, −K +Na conditions led to more rapid loss of reserves during the earlier part of the period studied than was true under +K −Na conditions. This may have been merely a response to the greater respiratory rate of the −K +Na plants. It is thought, however, that such an explanation fails to account for the larger amount of carbohydrates in the −K +Na plants. It seems that there may be a direct effect of −K +Na nutrition to increase the lipolytic rate in the cotyledons. A hypothesis might be advanced that potassium plays a specific role in protecting the lipid reserves from the lipolytic enzymes and that, when sodium is substituted for potassium, either (*a*) the permeability to the enzymes of the membrane surrounding the lipid droplets is lowered, or (*b*) the surface per gram of lipid is so greatly increased owing to the lowering of the interfacial tension between the fats and the protoplasm that much more rapid destruction

of the fats by lipase results, or (*c*) both. Modification of the permeability of membranes surrounding an enzymatic substrate has been suggested by COVILLE (**2**) to account for the change of starch from a state of immunity to one of vulnerability toward diastase, or the reverse, in the continuous presence of that enzyme.

The reasoning in regard to the relationship of sodium and potassium to the protection of the lipid substrate from esterases is supported by the work of CLOWES and of HARKINS and ZOLLMAN as described by GORTNER (**6**). These workers showed that sodium chloride in solution greatly increased the surface of a given quantity of olive or paraffin oil dropped into the solution by lowering the interfacial tension of the system, while calcium chloride exerted the opposite effect. It will be noted that the −K −Na plants, which were grown with a slight excess of calcium in comparison with the other two treatments, retained more lipid in the cotyledons than either of the other two groups of plants both at 7 and at 15 days after planting.

Lipolysis may be so rapid under certain circumstances (experiment II) that carbohydrates originating from the breakdown are "forced" into a storage state by mass action; or, on the other hand, it may proceed somewhat more slowly (experiment III) or may be exceeded by an exalted respiratory rate to such an extent that appreciable storage carbohydrates do not result. It is thought that this is a pertinent consideration in attempting to explain the failure of experiment III to duplicate the trends in carbohydrate distribution shown by experiment II.

In explaining the occurrence of more lipid in the −K +Na cotyledons at 15 days than in the +K −Na cotyledons (the reverse of the situation at 7 days), two suggestions may be made. One is that prolonged potassium starvation resulted in malfunctioning of the translocating mechanism specifically with respect to the products of lipid destruction and that this depressed the hydrolysis of storage fats toward the end of the experimental period. There was evidence of the accumulation of carbohydrates in the cotyledons of the −K −Na plants of experiment III, which might possibly be considered as owing to interference with their translocation. Such an explanation would not only be contrary to the conclusions of NIGHTINGALE *et al.* (**15**), who found translocation unimpeded under −K conditions, but would also be at variance with the facts that (*a*) no anatomical differences in phloem structure between treatments was evident and that (*b*) there was no interference with amino acid translocation. Invocation of any such explanation would thus imply a translocation mechanism of great specificity, in which the transport of one class of substances normally transported could be impeded independently of other classes of substances. On the other hand, slight reduction of the cross-sectional area of phloem tissue has been reported by WATTS (**26**) to accompany potassium deficiency in the seedling tomato, and it does not seem impossible that a certain amount of impedance or malfunctioning of the translocation tissue could exist before it becomes evident microscopically.

The preferable explanation seems to be that the reversal of rate of utilization of lipid reserves between treatments was bound up in a manner unknown at present with the radical change in morphology and physiology of the developing seedling which intervened between the

7th and 15th days (see above, "Dry matter").

Summary

1. Virginia peanuts were grown in the dark in pots of quartz sand and were watered daily or every second day with either a complete nutrient solution (+K −Na), a solution in which sodium had been substituted for potassium (−K +Na), or one in which neither sodium nor potassium was present (−K −Na).

2. Cotyledons were harvested at the 6th or 7th and again at the 15th day after planting, were counted, weighed, and analyzed for reducing and total sugars, starch and dextrins, acid-hydrolyzable substances, soluble and insoluble nitrogen, and total lipids.

3. At 7 days carbohydrates were more abundant and lipids and crude protein less abundant in the −K +Na than in the +K −Na cotyledons. At 15 days the lipid relationship had reversed itself. Plants grown with −K −Na solution had somewhat more lipid material in their cotyledons at each stage than either of the other groups.

4. Determinations of lipolytic activity and histological study of the vascular structure of the cotyledonary petiole revealed no differences between treatments at 7 or 15 days.

5. The total dry weight of plants at both 7 and 15 days was less for −K +Na than for +K −Na plants; this is interpreted as indicating a higher respiratory rate owing to potassium deficiency.

6. The more rapid utilization of protein under −K +Na conditions is probably a reaction to the low availability of potassium for the catalysis of nitrate reduction. Since the latter condition limits the opportunity for the formation of amino acids in the −K plants, such new protein as is formed in the developing seedling axis must come from resynthesis of the proteins withdrawn from the reserve areas.

7. Histological preparations revealed the presence of several times as much starch in the seedling axis of the −K plants as in the controls. This appears to be confirmatory of the seedling's inability to utilize carbohydrate in protein synthesis.

8. No definite evidence of interference with translocation under −K conditions was found.

9. It is suggested that potassium may have a specific role in protecting the lipid substrate from lipase.

10. Additional evidence was obtained that sodium may to a certain extent alleviate potassium deficiency.

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DEVELOPMENT OF THE EMBRYO OF THYMELAEA ARVENSIS

J. VENKATESWARLU

Introduction

Although the Thymelaeaceae have received considerable attention from morphologists, no accurate and detailed account of the development of the embryo of any species in the family is available. Therefore, the writer undertook an embryological study of *Thymelaea arvensis* Lamk. and published some observations elsewhere (10). Since then, he has had the benefit of certain suggestions from Dr. D. A. JOHANSEN, in the light of which it became desirable to make a revised study of the embryogeny of this species. The present paper embodies the results and includes some new illustrations. After the present investigation was completed, the writer came across the paper by SOUÈGES (9) on *Daphne mezereum*.

Material and methods

The material was collected from plants growing at Sopore, Kashmir, India, and was fixed in formalin–aceto-alcohol by Professor A. C. Joshi. After imbedding in paraffin, sections were cut 10–14 μ thick and stained in Heidenhain's iron hematoxylin.

Investigation

The first division of the zygote is transverse (fig. 1) and occurs after a few endosperm nuclei are formed. Very soon a three-celled proembryo is evident. Since the division leading to this stage was not observed, it is not possible to state definitely whether the middle cell is derived from the basal or the terminal cell of the two-celled proembryo. Fuchs (3), who studied some early stages of embryo development in *Daphne laureola*, stated that the middle cell is derived from the terminal cell of the two-celled proembryo. In *D. mezereum*, however, the middle cell is derived from the basal cell according to Souèges (9).

TABLE 1

Origin of parts of mature embryo from specific cells of proembryo

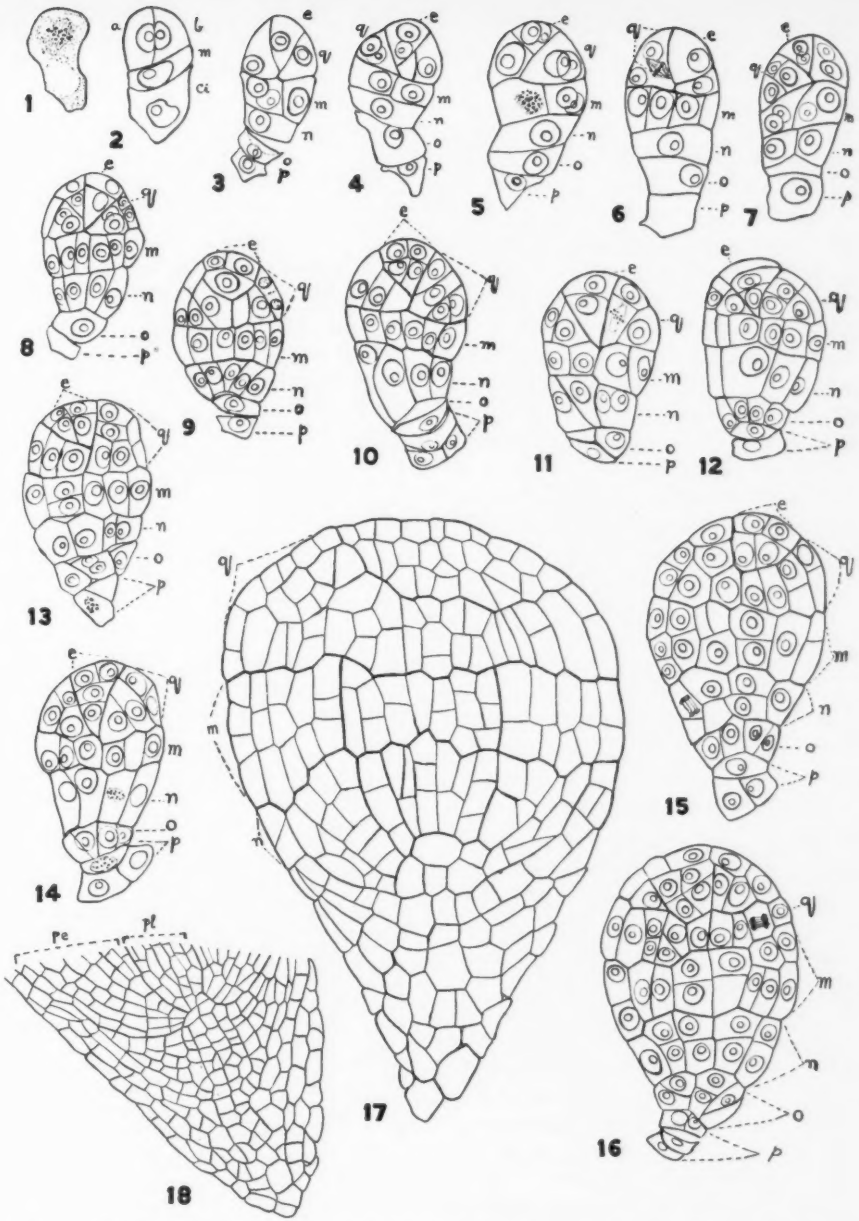
Cells of proembryo	Derivative
e	Stem apex
ct	
q	Cotyledons
m	Hypocotyl
n	Major portion of root
o	Root apex and root cap
p	Suspensor

For convenience of description the cells of the three-celled proembryo are designated as follows: the cell nearest the micropyle, *ci;* the middle cell, *m;* and the terminal cell, *ct*.

First the terminal cell, *ct*, divides by a slightly oblique wall into two juxtaposed cells, *a* and *b*, one of them slightly smaller than the other (fig. 2). The next wall is erected in the larger of these two cells and is diagonal. The upper daughter cell arising out of this division is triangular in outline and forms the epiphyseal initial in the terminal tier. The middle cell, *m*, undergoes two longitudinal divisions in which the vertical walls are arranged perpendicularly to each other. The four cells resulting are arranged in a circumaxial manner (figs. 3, 4, 5, 7). The cell *ci* also undergoes a transverse division, producing two superposed cells, *n* and n^1. The latter of these divides transversely to form two superposed cells, *o* and *p* (figs. 3, 4, 5, 6).

The relation of the different organs of the mature embryo to specific cells of the proembryo is shown in table 1.

Segmentation in the various tiers of the proembryo proceeds in a basipetal order. The epiphyseal initial, *e*, first divides by a vertical wall (figs. 4, 5, 7). The daughter cells undergo periclinal divisions, as a result of which the epidermal initials in the region of the stem apex are differentiated. The inner cells undergo a few more divisions. The derivatives of the epiphyseal initial are perceivable as a distinct group of cells (figs. 10, 15–17), the epiphyseal region, which constitutes the stem apex. The three subepiphyseal cells, *q* (fig. 5), first divide periclinally to differentiate the epidermal initials in that region. The derivatives of the subepiphyseal cells form the cotyledons of the mature embryo (figs. 6–17). The four cells of tier *m* are first divided longitudinally (fig. 6). Periclinal divisions take place and result in the differentiation of the epidermal initials in this tier (fig. 8). The inner cells also divide longitudinally and transversely (figs. 13, 15, 16). Differentiation into the three histogenic layers occurs in this tier before the appearance of the cotyledonary pro-



FIGS. 1–18.—Stages in the development of embryo of *Thymelaea arvensis*. *a*, *b*, daughter cells formed from terminal cell (*ct*) of proembryo; *m*, middle cell of proembryo; *ci*, basal cell of three-celled proembryo; *q*, cotyledon-forming region of terminal tier; *n*, upper daughter cell formed from first division (transverse) of *ci*; *o*, *p*, upper and lower daughter cells formed from n^1 (lower daughter cell of *ci*); *pe*, periblem; *pl*, plerome. Figs. 1–17, ×100; fig. 18, ×222.

tuberances in the subepiphyseal region (fig. 17). Segmentation of the cells in the tier formed by cell *n* follows that in *m* and is similar. The derivatives of *n* (the upper daughter cell derived from the first division of cell *ci*) form the major part of the root. Cell *o* (the upper daughter cell arising from the first transverse division of n^1) divides twice longitudinally to form four circumaxially arranged cells (the hypophysis). Tangential divisions take place in these and result in the formation of an inner and an outer layer of cells (fig. 16). The inner cells undergo transverse divisions and produce the two layers of cortical initials of the root apex. The outer layers, by further periclinal and anticlinal divisions, give rise to the root cap. Cell *p* (the lower daughter cell arising out of the transverse division of n^1) divides once or twice transversely and forms a short suspensor, two or three cells in length (figs. 10, 12–16). One of the cells of the suspensor may undergo a longitudinal division (figs. 10, 14–16).

Discussion

The observations show clearly that in the embryogeny of *T. arvensis* derivatives of both the terminal and the basal cells of the two-celled proembryo contribute to the formation of the embryo proper. This is characteristic of the Asterad Type of embryo development. JOHANSEN[1] (5), for the first time, recognizes some variations under each type of embryo development. Under the Asterad Type, that of *Erodium cicutarium* described by SOUÈGES (7) is recognized as the Erodium Variation. This is characterized by the formation of an epiphysis in the terminal tier and the presence of a suspensor. *T. arvensis* is embryonomically similar to *Erodium cicutarium* and keys out to the same variation.

SOUÈGES (9) worked out in detail the development of the embryo of *D. mezereum*. In this species no epiphyseal initial is delimited, in contrast to the situation in *T. arvensis*. The basal cell (*cb*) of the two-celled proembryo divides first by a vertical or slightly oblique wall into two juxtaposed cells (*m* and *ci*). In *T. arvensis*, however, the basal cell (*ci*) and the middle cell (*m*) of the three-celled proembryo are superposed in their positions. In *D. mezereum*, *m* and *ci* divide further. The former cell segments by a vertical wall, and the latter by an oblique wall in the horizontal plane to give rise to two daughter cells termed *n* and n^1. The derivatives of *m* and *n* contribute to the formation of the hypocotyledonary region, and those of n^1 produce the root apex. No suspensor is formed. The embryogeny of *D. mezereum* thus follows the Asterad Type. However, the development of the embryo of *D. mezereum* differs from that of *T. arvensis* in the absence of an epiphysis and in the nonformation of a suspensor. Both these features are characteristic of the Penaea Variation of the Asterad Type, and the case of *D. mezereum* keys out to this variation.

In the ENGLER system of classification (2) the Thymelaeaceae, Penaeaceae, Geissolomataceae, Elaeagnaceae, and Oliniaceae are included in the subseries Thymelaeineae under the series Myrtiflorae. BENTHAM and HOOKER (1) removed the Thymelaeaceae, Penaeaceae (including the Geissolomataceae), and Elaeagnaceae to the Monochlamydeae, in which they were placed together with Laurineae and Proteaceae in the series Daphnales. HUTCHINSON (4) made a departure from both the above arrange-

[1] Dr. D. A. JOHANSEN kindly furnished relevant information relating to the variations of the Asterad Type of embryo development and about our present knowledge of the embryology of the Myrtales.

ments and erected a separate order, Thymelaeales, under which he brought together the Thymelaeaceae, Penaeaceae, Geissolomataceae, and Nyctaginaceae, the last family being included by most systematists in the order Centrospermales. The rest of the families of the Myrtiflorae (of Engler's system) are divided into two orders, the Lythrales and Myrtales. According to ENGLER, the Thymelaeales were derived from the Caryophyllales through the Lythrales.

Embryo development in the Lythraceae, Sonneratiaceae, Onagraceae, and Myrtaceae takes place according to the Onagrad Type (the outmoded Capsella Type). According to JOHANSEN (5), the embryo development in *Rhizophora mangle* "is said 'to be probably of the Capsella Type,' though the evidence is unconvincing"; in the Melastomaceae "it is probably according to the Solanad Type." In the rest of the families included under the subseries Myrtineae in the ENGLER system, our knowledge of the embryogeny is in an unsatisfactory condition. The writer has recently studied the development of the embryo of *Poivrea coccinea*, one of the Combretaceae, and found it to conform to the Erodium Variation of the Asterad Type. In the Penaeaceae and Geissolomataceae, which are included in the subseries Thymelaeineae, the embryo development keys out to the Penaea Variation, Asterad Type. In this variation the embryo is globular and no suspensor is formed.

For the Nyctaginaceae the embryo development was described in *Oxybaphus viscosus* by SOUÈGES (8) and in *Boerhaavia diffusa* and *B. repanda* by KAJALE (6). It takes place according to the Polygonum Variation, Asterad Type. In this variation a suspensor is formed, but no epiphysis is differentiated.

Considering the above facts of embryogeny in the various families with which the Thymelaeaceae have been held to be related, the present study supports a close alliance between the Thymelaeaceae and Combretaceae, on the one hand, and also with the Geissolomataceae and Penaeaceae, on the other. Further, it also supports the grouping-together of the Thymelaeaceae, Geissolomataceae, Penaeaceae, and Nyctaginaceae as proposed by HUTCHINSON, since in all these families the Asterad Type of embryo development takes place, the difference between them being only one of variation. Even within the family Thymelaeaceae two variations of the Asterad Type seem to occur. As such, the differences in variation do not seem to be of primary importance in phylogenetic considerations.

It may also be mentioned that the Nyctaginaceae differ from the other families included in the order Centrospermales in that embryo development is of the Asterad Type. Hence the embryological evidence does not favor its placement in the Centrospermales.

Summary

1. The embryo development of *Thymelaea arvensis* has been described in detail. It conforms to the Erodium Variation, Asterad Type.

2. A comparison of the embryogeny of the Thymelaeaceae with allied families supports a relationship between that family and the Combretaceae, on the one hand, and the Thymelaeaceae, Geissolomataceae, and Penaeaceae, on the other. The development of the embryo in the Nyctaginaceae differs from that in the rest of the Centrospermales and resembles that of the above-mentioned families. Consequently, HUTCHINSON's action in placing the Nyctaginaceae together with the Thymelaeaceae, Penaea-

ceae, and Geissolomataceae is favored by the embryogenic evidence.

The writer is grateful to Dr. D. A. JOHANSEN of Pomona, California, for his kind interest, critical suggestions during this investigation, and help in the preparation of the paper. He is also thankful to Professor A. C. JOSHI, Government College, Lahore, India, for the material.

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CURRENT LITERATURE

A Botanist in Southern Africa. By JOHN HUTCHINSON. Foreword by JOHN C. SMUTS. London: P. R. Gawthorn, Ltd., 1946. Pp. 686 + 49 pls. £2 5s.

Modern scientific writing is often impersonal in character and presentation. It is therefore a refreshing experience to read the delightful and highly informative travelogue of HUTCHINSON's expeditions in southern and central Africa, including a trip to Lake Tanganyika as guest of General SMUTS in 1930. In fact, the responsive reader will be tempted to pack his things at once and start out on his own expedition to this botanical El Dorado. Enthusiasm for the rich and extraordinary plant life of southern Africa coupled with a fine sense of humor pervades the book, as the narrative progresses from day to day and place to place, often following the tracks of early explorers. The numerous photographs, drawings, and maps add materially to the enjoyment of reading and the botanical instruction of those unable to visit Africa. HUTCHINSON's modest appraisal of his own product as a "*pot pouri* of my impressions and expressions on a botanical tour in South Africa with an account of the plants that I saw on the way" covers only part of the book but does not indicate the many observations and new ideas he managed to crowd into it.

Thus, the beginning chapters (1, 2, 8), devoted to general discussions, disclose HUTCHINSON's unreserved acceptance of WEGENER's displacement theory as the explanation of the origin and affinities of the flora of southern Africa. Some of the closing chapters (29–31) represent admirable summaries of early botanical work in South Africa, of botanical literature pertaining to its flora, and of the floral regions generally distinguished. The author's own collections are recorded at the end of each chapter and rearranged in chronological sequence in chapter 34 at the end of the book. As counted by the reviewer, descriptions and accounts of fifty-two new or inadequately known species, and new combinations as well as one new composite genus (*Zoutpansbergia*), are provided in the abundant footnotes.

Using his broad experience gained through prolonged occupation with the flora of tropical Africa and his newly acquired knowledge of the flora of southern Africa, HUTCHINSON prepared chapter 32 on "A New Phylogenetic Arrangement of the Families of Gymnosperms and Flowering Plants Represented in South Africa." This treatment supersedes in many respects his well-known two-volume set entitled *Families of Flowering Plants.* Although some "basic" groups such as Magnoliaceae, Dilleniaceae, etc., are missing in South Africa, they are indicated at least by name in brackets in order to show the entire system as now arranged by HUTCHINSON. Some significant changes concern the dissolution of the Gamopetalae and the disposition of its former members among their nearest relatives in the Archichlamydeae. The distinction of two phyla, viz., *Lignosae* and *Herbaceae* in the Dicotyledones, foreshadowed in his earlier work, is here carried to its logical conclusion, separating the "one fundamentally and remaining predominantly woody" from "the other fundamentally and remaining predominantly herbaceous." For, in the author's opinion, "the woody and herbaceous branches are considered to have been evolved side by side, the woody being probably the older." Although all stocks and their probable sources of derivation are listed in consecutive order—the pages must be read from the bottom up—the appended chart (in color) readily transmits the concepts and arrangements proposed in the text. At this point the reader is inclined to agree with HUTCHINSON's statement that "the more truly natural a system becomes the more difficult it is to express on paper." The chart also shows other changes made, viz., that the Araliales (Cornaceae and Araliaceae) are "here regarded as representing a parallelism with Umbelliferae" and that the grasses appear as the "most advanced of all" Monocotyledones.

These examples clearly indicate the great importance of this book to the botanist as a source of information and new concepts beyond the great personal pleasure awaiting him on reading it. Its usefulness could have been enhanced by the inclusion of an index to plant names, localities, and persons. The price appears reasonable in view of the excellence of its physical makeup and the fine quality of paper used.—THEODOR JUST, *Chicago Natural History Museum.*

Pflanzliche Infektionslehre: Lehrbuch der allgemeinen Pflanzenpathologie für Biologen, Förster und Pflanzenzüchter. By ERNST GÄUMANN. Basel: Verlag Birkhäuser, 1945. Pp. 611+311 figs.+90 tables.

The book under review had its origin in lectures delivered during the last 20 years by Professor GÄUMANN to his classes at the Eidgenössichen Technische Hochschule in Zürich. It is designed to acquaint the reader with the theory of plant infections, i.e., "with some biological problems which lie at the basis of the diseased state, rather than with special plant pathology" (the knowledge of individual diseases) and so "to supplement plant pathological instruction on its theoretical side."

To accomplish these ends, GÄUMANN presents the parasitological and epidemiological points of view by means of selected examples and delimits the technical terms, some of which, even now, have become part of daily usage. Since many of these terms had their origin in medicine and have experienced shifts in meaning as they entered the botanical field, deviations between phytopathological and medical thinking are indicated in the book. As a result the volume contains many comparative discussions of the theoretical aspects of infectious diseases in animals and plants. Although GÄUMANN is aware that animals and flowering plants are capable of acting as disease incitants in plants, and the book contains much interesting material bearing on the mistletoe diseases of plants, he restricts its scope to "viroses, bacterioses, and mycoses," which he designates "true infectious diseases." In spite of this limitation of its scope, and of the author's awareness of the existence of a vast domain of plant diseases in which infects play no causative role whatsoever, the book carries the subtitle "Textbook of General Plant Pathology." Also, it is surprising that, while the author states that "every disease rests primarily on a functional disturbance of the organism," he nevertheless uses the tautological expression "physiological disease" to designate the diseases not incited by infects. The book approaches a true general plant pathology in two sections of chapter 5. One of these sections deals with the morphological-anatomical manifestations of infectious diseases; the other, with their physiological manifestations. Both sections are very well done.

GÄUMANN's success in pointing out deviations between medical and phytopathological thinking reveals the inadequacy of much of the terminology and of many of the concepts current in theoretical thinking about infectious diseases. This inadequacy is especially evident in the section dealing with the responses designated "defense reactions," including the phenomena of immunization and of immunity.

GÄUMANN envisages the complicated relations between infect and host in an infectious disease as a battle and has frequent recourse to the language of conscious purposive behavior to describe and to explain the complex interplay of the activities of infect and of host. Also, in chapter 5, one reads: "An infectious disease is more than the mere reactions of the host to the infect; it forms an independent process, and independent biological complex. . . . . Incitant and host unite themselves, after infection has been established, into a life community of a higher order which is more than their mere summation."

The vast subject matter of the 571 closely printed pages of text is presented in an excellent introduction and in six chapters with the following headings: "Infection," "Infect Chains (Infektketten)," "The Parasitic Qualifications of Incitants (Die parasitische Eignung der Erreger)," "Disease Susceptibility of Hosts (Die Krankheitsbereitschaft der Wirte)," "Disease (Die Erkrankung)," and "Control (*Bekämpfung*) of Infectious Plant Diseases." There is an excellent and detailed table of contents. The literature citations are grouped in ten pages at the close of the book, and there is an index.

The book reveals an amazing mastery of the enormously diverse subject matter which is presented interestingly and effectively. There is a wealth of good graphs, photographs, drawings, tables, and maps. The volume is a contribution of the first order, and, although a textbook and not a manual, it nevertheless is a veritable mine of pertinent factual material organized on a theoretical basis.

Printing and binding are an achievement under postwar conditions. The book is an example of intellectual vigor and activity in the midst of terrific distractions. Every botanist interested in infection and infectious disease in plants will find it profitable to consult and to study this book. Finally, the book indicates that GÄUMANN's students are the beneficiaries of an unusually high level of phytopathological instruction.—GEORGE K. K. LINK.

General Horticulture. By THOMAS J. TALBERT. Philadelphia: Lea & Febiger, 1946. Pp. 452. Illus. $4.00.

This volume aims to present a "clear and concise treatise on general horticulture" for undergraduates in schools and colleges and for progressive producers. In this it succeeds in so far as north temperate deciduous fruits are concerned, but it might properly be considered as failing in its objective by one seeking full information on vegetable crops and ornamental horticulture. This situation comes about by virtue of the fact that TALBERT has used *Fruit Crops*, published in 1939, as the basis for *General Horticulture*, adding five chapters to this re-written text in order to broaden its scope. There are approximately 320 pages devoted to deciduous north temperate fruits, 20 pages to vegetable-growing, 25 pages to beautifying the home grounds, 25 pages to nut-tree culture, 30 pages to tropical and subtropical fruits, and 20 pages to handling and distributing horticultural crops.

TALBERT has succeeded in including a wide range of fundamental information, concisely stated, with selected references at the end of each chapter for those who may seek an elaboration on particular topics. He has succeeded also in tying these fundamentals to the practical problems of horticulture, drawn from his wide experience from field contacts, fruit-growers' meetings, and conferences. The result is a book with a distinctly horticultural point of view that should be found very useful in north temperate regions where the emphasis is placed upon fruits.—H. B. TUKEY.

The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat. By K. STARR CHESTER. Waltham, Mass.: Chronica Botanica Co.; New York: Stechert-Hafner, Inc., 1946. Pp. xvi+269. $5.00.

As indicated by its title, this publication deals primarily with the leaf rust of wheat, which CHESTER chooses to consider as a species, *Puccinia triticina* Erikss. The Introduction gives a brief summary of the history of the disease and the names applied to it. In following chapters the origin, distribution, and economic importance of the rust and its effect on its hosts are discussed. The importance of the leaf rust as an economic factor has only recently been understood. CHESTER points out not only that it may be very important in epiphytotic seasons, such as 1938, when a loss of more than 100,000,000 bushels of wheat occurred in the United States, but that the fairly regular general drain on the profits of agriculture may be even more important.

A summary of the information concerning the morphology, physiology, and cytology is given. The heteroecism of the rust and the role of the aecial stage are discussed. Two chapters are devoted to physiologic specialization. The reactions of the varieties of wheat differentiating the physiologic races of the rust are described. The effects of environmental factors on these reactions are discussed, and it is concluded that the reactions of the varieties Carina, Brevit, and Hussar are so unstable that accurate separation of physiologic races of the rust by their reactions is not possible. It is recommended that their use as differential varieties should be discontinued, and a reclassification of the physiologic races of the rust is given.

Two chapters are devoted to the factors affecting rust survival and development. The effect of humidity, temperature, and light upon spore germination, infection, and development of the rust through effects on its hosts are discussed. The importance of the hibernation and aestivation in the uredinial stage is emphasized. CHESTER presents evidence that there is a "critical month" in the spring, that having a mean temperature close to 50° F., which determines the intensity of the development of leaf rust. Under the heading "rust dissemination" he reviews the production of spores, wind dispersal, rate of fall, distance carried, and insect and seed carriage. Annual cycles in the development of the rust in various parts of the world and the possibility of prediction of epiphytotics are discussed. Four chapters are given to control. The factors which tend to limit the development are described, and the results which have been obtained with fungicides are summarized. Considerable space is devoted to a discussion of control through the use of rust-resistant varieties and of the methods used in breeding them.

As CHESTER has stated in the Introduction, the researches of the past quarter-century have added much to our knowledge of the nature and prevention of the cereal rusts, and there is need for a comprehensive treatment of them. Although this book is for the most part a monographic treatment of only one of the cereal rusts, they have much in common, and the volume affords a very useful reference for teachers and investigators in the fields of plant pathology and agronomy.—E. B. MAINS.

A Textbook of Systematic Botany. By DEANE B. SWINGLE. 3d ed. New York: McGraw-Hill Book Co., 1946. Pp. xv+343. $3.50.

Based on teaching experiences of 15 years and on solicited suggestions from others, the late Dean SWINGLE has synthesized and balanced the diverse elements of the oldest of botanical sciences into a pattern which is modern in approach and pedagogical in form.

A short statement on the meaning of systematic botany precedes the Introduction. The latter includes interesting facts about TORREY, GRAY, BESSEY, and COULTER and points out the usefulness of taxonomy and nomenclature in forestry, agriculture, horticulture, floriculture, range management, ecology, and paleobotany. The relation of evolution to taxonomy is presented in a most readable chapter.

Indented and parallel keys are taken from well-known floras and analyzed, and floral diagrams and floral formulas are explained and used throughout the text. Field and herbarium methods of preparing plants for further reference are discussed. Nouns and adjectives used to describe parts of plants, together with their meaning, are arranged according to plant organs.

One chapter is devoted to gymnosperms; 125 pages to the flowering plants. Fifty-seven families of dicotyledons and monocotyledons are arranged according to the system of BESSEY. Information for each family includes a general description, often a photograph or a drawing of selected plants, an emphasis on distinguishing characteristics of the plant organs, floral diagrams and formulas, and a chart for some families.

The value of the "type concept," the progress made by successive Botanical Congresses, and the principles of classification are treated from a broad and unbiased approach. The experimental method in taxonomy is discussed briefly. Historical aspects of systematic botany, which have occupied so much space in textbooks, are told in a concise and interesting manner. Useful information is given concerning the literature of systematic botany. Floras are named according to regions, and reference books for other aspects of systematics are discussed.

The present edition will have a wide appeal to those who seek a general education beyond the level of elementary botany and to the many teachers and students of systematics who will find this text adaptable to great variations in flora and curriculum.—PAUL D. VOTH.

The Genus Bazzania *in Central and South America.* By MARGARET FULFORD. "Annales Cryptogamici et Phytopathologici," Vol. 3. Waltham, Mass.: Chronica Botanica Co.; New York: G. E. Stechert & Co., 1946. Pp. xv+175. Figs. 59. $5.00.

A thorough review of even the more common leafy liverworts of the Western Hemisphere has long been a pressing need. In 1936 Miss FULFORD completed the monographic study of the species of *Bazzania* which have been collected in the United States and Canada (American Midland Naturalist 11:385–424). The collating of collections from the neighbor-nations to the south of us has resulted in the impressive monograph just published.

Following a discussion of previous arrangements of species within the genus *Bazzania*, a natural grouping under two subgenera is achieved by FULFORD. Seven species possess two-toothed leaves, while thirty-nine (one a variety) are tridentate. Of the five sections which comprise the second subgenus, only one is not represented in this hemisphere. Of the 122 species studied, 71 are reduced to synonymy. Thus much of the confusion created by STEPHANI and other early bryologists has been cleared away. *Herpetium* and *Mastigobryum* are considered synonyms of *Bazzania*. This further simplifies the taxonomy of these liverworts, thereby saving the time of the professional bryologist and lessening the frustration of the amateur.

Dr. FULFORD herself executed the excellent line drawings for each species. Leaf arrangement, denticulation of leaf margins, and thickenings of the cell wall are drawn to scale. For each species all available synonyms are cited, a complete description of the plant is given, habitats are specified, erroneous reports are discussed, collection localities are listed, and all literature references are named. The work is complete from diagnostic keys to index.

Only about twenty-one additional species or varieties were not available for study because of the unsettled conditions during the war. Many of these names will undoubtedly be adjudged synonyms.

This volume sets a high standard in research, in editing, and in publication.—PAUL D. VOTH.

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